ABSTRACTS of SFRR-INTERNATIONAL 2021 VIRTUAL MEETING

Keynote and Award Lectures

Trevor Slater Award Lecture
A redox-centred view of skeletal muscle responses to exercise and ageing

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Increased generation of reactive species by contracting skeletal muscle was first recognised in the 1980's and these observations sparked a great deal of interest in the topic that focussed on the potential damaging effects of contraction-induced free radicals and reactive oxygen species. This initial work was rapidly followed by identification of the key molecules generated, superoxide and nitric oxide, and the sites for their generation in contracting muscle. A number of pivotal studies subsequently recognised that this increased generation plays an important physiological role in signalling essential adaptations to maintain muscle homeostasis during exercise prompting the extensive current research activity aimed at elucidating redox-regulated responses to contractile activity in muscle. The number of exercise-induced adaptations that are mediated by redox-dependent processes and responsive to increased reactive oxygen species continues to expand and the pathways identified now include stress responses, increased mitochondrial biogenesis, post-exercise muscle glucose uptake and inflammatory responses. Hydrogen peroxide generated by a plasma/T-tubule membrane-located signalling NADPH oxidase appears to be the key mediator of these changes, but the detailed signalling mechanisms involved remain poorly defined. Ageing leads to an attenuation in the efficacy of several of these redox-regulated processes which contributes to a reduced ability to maintain muscle mass and function in older individuals. Full elucidation of these pathways therefore holds great promise as an approach by which to identify targeted interventions that will maximise the beneficial effects of exercise and help maintain skeletal muscle mass and function in the elderly.

Keynote Lecture – 1
A mitochondrial etiology of complex diseases

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The mitochondrial genome consists of hundreds of mitochondrial DNAs (mtDNA) plus one to two thousand nuclear DNA (nDNA) genes. To prove that mtDNA mutations can cause common disease phenotypes, we introduced discrete mtDNA mutations into the mouse female germline. An mtDNA COI^{V421A} mutation causes reduced complex IV and cardiomyopathy and diabetes. An $ND6^{P25L}$ mutation causes reduced complex I, increased reactive oxygen species (ROS), and neurodegenerative disease. Simply mixing NZB and 129 mouse mtDNAs causes long term memory defects. The mtDNA genotype and associated ROS production regulates the gut microbiome.
More severe mtDNA mutations are heteroplasmic (mixed mutant and normal mtDNAs) and can present with a spectrum of phenotypes. For the $tRNA^{Leu(UUR)}$ m.3243A>G mutation, ~20-40% 3243G mutant can present with diabetes, ~50-80%

3243G with neuromuscular and cardiac manifestations, and ~90-100% 3243G with lethal pediatric disease. Analysis of transmitochondrial cybrids with different percentages of the 3243G mutation revealed that each clinically discrete heteroplasmic range has a distinctive nDNA and mtDNA transcription profile determined by mitochondrial metabolites, mitochondrial and nuclear redox states, and ~150 discrete histone modifications.
mtDNA haplogroups arose as women migrated out-of-Africa and encountered regional environments that selected for functional mtDNA variants. These haplogroups now predispose to a wide range of common disease phenotypes when encountering new environments. mtDNA haplogroups can also modulate the clinical manifestations of nDNA gene mutations. Null mutations in the heart-muscle-brain isoform of the adenine nucleotide translocator (*ANT1*) are associated with hypertrophic cardiomyopathy on mtDNA haplogroup H or dilated cardiomyopathy on haplogroup U. Combining our mouse *Ant1* null mutant with our COI^{V421A} and $ND6^{P25L}$ mtDNA cause similar phenotypic alterations.
The accumulation of somatic mtDNA mutations are associated with aging and the delayed onset and progressive course of diseases. Thus, the mitochondria are the integrators of the effects of the environment and nDNA and mtDNA variation.

Keynote Lecture – 2
Oxidative eustress and oxidative distress

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In the aerobic living cell, a steady flux of oxidants is generated from sources under control of endogenous metabolic cues such as growth factors or cytokines, and also by exogenous signals. The physiological low exposure to oxidants is denoted *oxidative eustress*, it is essential in control of life processes through redox signaling and for maintenance of appropriate stress response capacity. Hydrogen peroxide is a central redox signaling molecule, maintained at low nM overall concentration intracellularly. Subcellular organelles (*i.e.* mitochondria, peroxisomes, endoplasmic reticulum, nucleus and the cytosol) operate at their particular setpoint level for H_2O_2 in redox signaling. The initial step in redox signaling is by reaction of H_2O_2 with particularly susceptible protein cysteine thiolates to form the corresponding sulfenates, causing changes in protein properties. Subsequently, protein kinase/phosphatase-dependent and other pathways are modulated. Stress response systems such as Nrf2 and NF-kB provide for adaptation, restoring redox balance. Supraphysiological exposure to H_2O_2 leads to non-specific oxidation of biomolecules and to disruption of redox signaling, called *oxidative distress*, which can lead to excessive stress responses, inflammation, autophagy and cell death. Fundamental life processes as well as metabolic disorders, diseases and aging have an oxidative stress component, an active field for research for a future redox medicine.

Keynote Lecture – 3
Reflections of an ageing free radical

Barry Halliwell*
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My interest in reactive oxygen species began during my D.Phil. in Botany at Oxford in 1971-73, during which I identified considerable production of H_2O_2 by plant organelles, and its involvement in metabolism. H_2O_2 is often argued as a cytotoxic molecule, but actually it is widespread in the environment, including in human urine, water and some of the beverages we drink. Indeed, reactive oxygen (ROS) and related species (RNS, RCS, RSS etc.) play key roles in Biology: they helped drive human evolution and they still shape our development from fertilization onwards. These concepts will be explained.
H_2O_2 is an important signaling molecule in many of these processes *in vivo*. It only becomes problematic when it encounters "catalytic" transition metal ions, whereupon much more reactive species such as hydroxyl radical ($OH^{\bullet}$) are formed. Such metal ions play key roles in neurodegenerative diseases, atherosclerosis and many other conditions. Indeed, an essential antioxidant defence is to sequester them in non-redox-active forms. Yet many antioxidants have failed in clinical studies. Reasons for this will be discussed and novel approaches presented, including an introduction to ergothioneine.

Lester Packer Award Lecture

Discovery of the KEAP1-NRF2 pathway regulating cellular response against oxidative and electrophilic stresses

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Transcription factor NRF2 is crucial for coordinated induction of cellular defense enzymes against oxidative and electrophilic stresses. KEAP1 acts as a sensor for these stresses and also acts as a subunit of ubiquitin-E3 ligase that degrades NRF2 constitutively. *Nrf2* gene knockout animals are sensitive to a wide variety of toxic electrophiles and reactive oxygen species, while *Keap1* gene knockdown animals show gain-of-function phenotype of the cytoprotection. Modifications of KEAP1 cysteine residues abrogate the ubiquitin ligase activity of KEAP1 and stabilize NRF2. This mechanism is referred to as the Cysteine Code. The Cysteine Code and Two-Site-Binding model between KEAP1 homodimer and single NRF2 molecule have been proposed for the regulation of the KEAP1-NRF2 system. Disruption of the Two-Site-Binding explains how the nuclear accumulation of NRF2 is attained in a KEAP1-dependent manner, providing solid basis for the development of drugs that induce NRF2. This mechanism is also referred to as the Hinge-Latch Model. Autophagy chaperon p62 is also found to disrupt the Hinge-Latch Model. Genetic as well as pharmacological induction of NRF2 nicely protect tissues from oxidative injury. Meanwhile, many somatic missense mutations have been identified in *KEAP1* and *NRF2* genes of human cancers. These mutations disrupt the KEAP1-NRF2 complex and result in constitutive activation of NRF2. The elevated expression of NRF2 target genes confers advantages on the growth of cancer cells through the metabolic reprogramming and induction of cellular defense enzyme. Researches on the KEAP1-NRF2 system are expanding, including NRF2 regulation of inflammation, metabolism, ageing and neuroprotection. New topics have been constitutively added to this field, such as hydrogen peroxide sensing by KEAP1, glycation of NRF2, heme metabolism related to NRF2-BACH1 pathway, space stress-mediated induction of NRF2, *etc*. Historical overview of the KEAP1-NRF2 system as well as recent progress in the KEAP1-NRF2 pathway will be discussed.

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Symposia

Symposium 1-1
Redox-stress response capacity decline and ER reductive stress in aging

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Aging is tightly associated with redox events. Using Caenorhabditis elegans and human fibroblasts as models, we compared the response capacity of young and old individuals to oxidative stress. In response to paraquat stress, young individuals generated more ROS and activated signaling pathways including p-ERK, p-AKT and p-AMPKα/β. After the initial response, young individuals then promoted NRF2 translocation and induced additional antioxidant enzymes and higher expression of phase II enzymes, including SOD, CAT, GPX, HO-1, GSTP-1, to maintain redox homeostasis. Moreover, young individuals also demonstrated a better ability to degrade damaged proteins by up-regulating the expression of chaperones and improving proteasome activity. Based on these data, we propose a new concept "Redox-stress Response Capacity (RRC)", which suggests cells or organisms are capable of generating dynamic redox responses to activate cellular signaling and maintain cellular homeostasis. The decay of RRC is the substantive characteristic of aging, which gives a new understand of the redox theory of aging.
As we know that redox homeostasis in cells is crucial for the function of biomacromolecules. Oxidative stress is known to mediate many cellular signal transductions in physiological and pathological processes, however, the biological effects and mechanisms of reductive stress (RS, an abnormal increase in electron pressure or reducing equivalents (GSH/GSSG; $NADH/NAD^+$; $NADPH/NADP^+$) are still poorly understood. Being different from the free-radical aging theory that oxidative damage leads to aging, we found that the ER exhibited reductive stress in senescent cells, and the reductive stress accelerated senescence. Further study showed that the decrease of Ero1α activity is the main cause of ER reductive stress and increasing the oxidative power in the ER successfully delayed aging. Our results suggest a new mechanism of aging which is insufficient oxidation in the ER and indicate a new strategy of anti-aging.

Symposium 1-2
Age and sex determine the effectiveness of redox adaptive homeostasis

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Both internal and external redox signals routinely modify patterns of gene expression via discrete signal transduction pathways. Several such signaling pathways are protective in nature. These include the Nuclear Respiratory Factor2 (NRF2) signaling pathway that can increase synthesis of the mitochondrial Lon protease via increased transcription and translation of the *lon* gene. The Lon protease maintains quality control of the mitochondrial proteome by eliminating damaged proteins, allowing replacement proteins to be synthesized. We have shown that normal levels of Lon maximize mitochondrial lifetime and minimize mitoptosis, whereas Lon down-regulation results in abnormal mitochondrial morphology and function and increased cell death.
Biological systems continuously make short-term adaptations both to set-points, and to the range of “normal” capacity, due to mild conditional changes, or to subtoxic, nondamaging levels of chemical agents. We have described this as “Adaptive Homeostasis,” which is defined as follows: “The transient expansion or contraction of the homeostatic range in response to exposure to sub-toxic, nondamaging, signaling molecules or events, or the removal or cessation of such molecules or events.” We find that adaptive homeostasis declines with age in organisms as diverse as worms, flies, and mammals, and decreases with senescence in mammalian cell cultures. We suggest that diminishing adaptive homeostasis may play a causal role as at least one significant factor responsible for the aging phenotype. Furthermore, although studies of humans, animals, and model organisms are often limited to a single sex, and cell culture studies may even be conducted with lines whose donor’s sex was unknown, our research has demonstrated distinct patterns of sexual dimorphism in adaptive homeostasis. Interestingly, although young males and females may exhibit dramatic differences in adaptive capacities and/or preferences, these distinctions are lost with age as adaptive homeostasis patterns converge.
Keywords: Mitochondria, redox regulation, adaptive homeostasis, sexual dimorphism

Symposium 1-3
Mitochondrial H_2O_2: new insights from imaging

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Mitochondria are the main oxygen consuming organelle in the cells, and historically have been considered as a principal intracellular source of ROS. This view has been supported by many studies performed on isolated mitochondria, which can produce substantial amounts of H_2O_2 in the presence of various inhibitors. When discussing mitochondrial ROS production, one of the most important aspects is a topology of the oxidant generation. Although experiments with isolated mitochondria clearly demonstrate the possibility of H_2O_2 diffusion from the matrix outside, it is not clear if this diffusion takes place in the context of the intact living cells. Would H_2O_2 be able to efficiently diffuse from the mitochondrial matrix to the cytosol of the intact living cells?
To answer this question, we performed a series of experiments using HyPer family probes and a chemogenetic substrate-controlled H_2O_2 generator, D-amino acid oxidase, targeted into the mitochondrial matrix. We demonstrate that although exogenous H_2O_2 can enter the matrix with approx. 5-fold concentration gradient, the oxidant produced in the matrix is not able to reach cytosol. Thioredoxin system appears to be responsible for this restriction, and the mitochondrial intermembrane space serves as an additional barrier for matrix H_2O_2 on its way to the cytosol.

Symposium 1-4
Mitochondrial transport and energy homeostasis in neuronal degeneration and regeneration

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Background: Mitochondria are cellular power plants that supply ATP for neuron growth, survival, and function. Neurons face the unique challenge of maintaining mitochondrial distribution and energy homeostasis in axons and synapses, where energy is in high demand. Chronic mitochondrial dysfunction is a central problem associated with neurodegenerative diseases. Removal of stressed mitochondria from axons/synapses constitutes a critical step of mitochondrial quality control. To survive an injury, neurons require high energy consumption to power various cellular events regeneration, thus recruiting mitochondria into injured axons helps.
Methods: We applied several challenging live imaging approaches, including adult neuron culture system from disease-onset mouse models, physical isolation of axons in microfluidic chamber system, super-resolution tools of axonal organelles interaction and dynamics, and gene rescue experiments in disease models.
Results: We identified syntaphilin as a static anchor for axonal mitochondria. Deleting *syntaphilin* robustly increases axonal mitochondrial motility *in vitro* and *in vivo*. Reduced mitochondrial motility and energy deficits in injured axons are one of intrinsic mechanisms controlling regenerative capacity. While mitochondrial transport progressively declines with neuron maturation, injury induces acute mitochondrial depolarization and ATP depletion. Enhancing mitochondrial transport rescues energy deficits in injured axons, thus facilitating regenerative capacity after spinal cord injury. We also demonstrated that stressed mitochondria are removed from axonal terminals triggered by the release of syntaphilin via mitochondria-derived cargos independent of Parkin, Drp1 and autophagy, thus maintaining axonal mitochondrial quality before activation of Parkin-mediated mitophagy.
Conclusion: Our studies provided new mechanistic insights into synaptic variability, energy deficits in CNS regeneration failure, and mitochondrial maintenance and quality control in axonal degeneration associated with chronic mitochondrial stress, thus broadly relevant to mitochondrial pathology and energy deficits in major neurodegenerative diseases and regeneration after injury and diseases.
Keywords: Axonal transport, energy deficits, mitochondrial stress, neurodegeneration, regeneration
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Symposium 2-1
Cellular redox signaling under physiological normoxia and ischemia-reperfusion

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In vivo, vascular and other cell types are exposed to physiological oxygen levels ranging from to ~2-13 kPa O_2, while cells cultured *in vitro* in standard CO_2 gassed incubators are routinely exposed to hyperoxic oxygen levels (18 kPa O_2). Although recent evidence highlights the importance of studying cellular redox signalling under physiological O_2 levels,

few studies have examined the effects of short- or long-term adaptation of cells to different O_2 levels (Keeley & Mann, Physiol. Reviews 2019;99:161-234). As molecular mechanisms regulating Nrf2 signaling have primarily been studied in cells exposed to hyperoxia, we have characterised Nrf2 gene targets in endothelial cells following 5d adaptation to 18 kPa, physiological normoxia (5 kPa) or hypoxia (1 kPa) in an O_2 regulated Scitive workstation. Gene profiling established that activation of Nrf2 and the induction of GSH-related genes were insensitive to alterations in O_2, whereas upregulation of HO-1 and NQO1 in response to electrophiles or NO was diminished under 5 kPa O_2 due to an upregulation of the Nrf2 repressor Bach1 (Chapple et al., FRBM 2016;92:152-62). We recently reported that a PP2A-mediated feedback mechanism regulates Ca^{2+}-dependent endothelial NO synthesis under 5 kPa O_2 (Keeley et al., FASEB J. 2017; 31, 5172–5183) and that enhanced SERCA activity under 5 kPa O_2 protects endothelial cells against calcium overload (Keeley et al., FASEB J. 2018; 32, 2531– 2538). Our findings provide the basis for a paradigm shift aimed at facilitating translation of findings under physiological conditions *in vitro* to disease pathology and the design of novel therapeutics.
Supported by British Heart Foundation and Heart Research UK.

Symposium 2-2
Transcriptomic and proteomic characterization of human cardiac progenitor cells

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Upon Acute Myocardial Infarction (AMI) and inherent Ischemia/Reperfusion (I/R) injury, endogenous cardiac progenitor cells (CPCs) are activated, contributing to myocardial repair through an auto/paracrine crosstalk between CPCs and cardiomyocytes (CMs) in stress. Transplantation of CPCs is being tested in clinical trials, and although improvements have been reported, the mechanisms of action of these cells are still mostly unknown.
Our work combines the development of I/R *in vitro* human cell models with advanced mass spectrometry proteomic tools to further characterize hCPC and unveil associated regenerative mechanisms. hCPCs employed in the clinical trial CARE-MI (NCT02439398) were used. Different strategies were explored to recapitulate I/R, including: use of human adult/mature cells, 3D culture and bioreactor technology. Firstly, we developed a transwell co-culture I/R model, with hCPCs and human induced pluripotent stem cell derived CMs (hiPSC-CMs). Following this work aiming at further improving the relevance of the I/R *in vitro* setup, 3D hiPSCCM cultures and bioreactors were combined, allowing the control/monitoring of critical environmental parameters.
Important features of I/R injury were successfully captured, including hiPSC-CM death, cell ultra-structure disruption, as well as increased release of inflammatory cytokines. hCPCs response to I/R was probed using whole proteome analysis (including quantitative SWATH-MS), allowing to propose new pathways in the hCPCs-mediated regenerative process along I/R injury. Our data shows that our AMI-setup up-regulates hCPC proteins associated with migratory, proliferation and stress response-related pathways. Moreover, our results reinforce the idea that paracrine-mediated mechanisms are central for hCPC activation, with the enrichment of several paracrine signaling pathways.

Symposium 2-3
Relevance of oxygen concentration in stem cell culture for regenerative medicine

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The key hallmark of stem cells is their ability to self-renew while keeping a differentiation potential. Intrinsic and extrinsic cell factors may contribute to a decline in these stem cell properties, and this is of the most importance when culturing them. One of these factors is oxygen concentration, which has been closely linked to the maintenance of stemness. The widely used environmental 21% O_2 concentration represents a hyperoxic non-physiological condition, which can impair stem cell behaviour by many mechanisms. The goal of this talk is to understand these mechanisms underlying the oxygen signalling pathways and their negatively-associated consequences. This may provide a rationale for culturing stem cells under physiological oxygen concentration for stem cell therapy success, in the field of tissue engineering and regenerative medicine.

Symposium 2-4
Do hypoxia mimetic agents' provide fidelity in replication of engineered oxygen control measures in human mesenchymal stem cell isolation and culture?

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Human bone marrow-derived mesenchymal stem cells (hMSCs) are in use in vast numbers of clinical trials globally. hMSC are multipotent, have a minimal immunophenotye, are a plastic adherent cell isolated from the bone marrow, and display an immunomodulatory paracrine exosome. The sinusoidal blood system of bone marrow results in a low physiological oxygen level. Further, defined reduced oxygen culture conditions enhance isolation and expansion of hMSC, a key requirement for hMSC-based cell therapies.
Engineered oxygen control measures provide controlled reduced oxygen culture environments to mimic a component of the marrow niche. We have evaluated the role of a panel of well-established Hypoxia Mimetic Agents (HMAs) ($CoCl_2$, DFO, DMOG) vs. 2% O_2 (Workstation + HypoxyCool media) in hMSC isolation and culture examining frequency of colony forming unit fibroblastic (CFU-F), differentiation, immunophenotype, nitroreductase activity (NA), reactive oxygen species (ROS), and mitochondrial copy number.
hMSC CFU-F recovery from primary bone was significantly inhibited in all conditions tested (21% O2, 21% O2 + HMA) vs. 2% O_2 ($p<0.05$). No significant gains were noted for 21% O_2 vs. 21% + HMA. As anticipated 2% O_2 culture resulted in significant increases in both ROS and NA and while $CoCl_2$ did not elevate ROS levels in 21%O_2 DFO, DMOG did to levels comparable to 2% O_2. $CoCl_2$ and DFO both failed to elevate NA levels in 21% O_2 while DMOG stimulated a 20% increase in NA activity. Finally, significant reductions of mitochondrial genome copy number were noted in 2% O_2 vs. 21% O_2 ($p<0.001$) and increases in 21% O_2 with all HMAs tested ($p<1X10^{-4}$) accompanied by compensatory alterations in mitochondrial activity.
In summary, HMAs do not provide an accurate biological replication of engineered oxygen control measures in hMSC culture and expansion. This is reflected in biological alterations which impact on cell yield, behaviour, and biology.

Symposium 3-1
New functions of selenoproteins: beyond redox reactivity

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Early research on selenium and selenoproteins has been focused on their direct roles in modulating redox status. This is because selenium has an electron configuration in the outermost shell that enables the element to have a variety of valences from -2 to +6, with the potential of giving or accepting electrons. Indeed, the first identified mammalian selenium-dependent protein was a peroxidase (glutathione peroxidase-1, GPX1) that use glutathione to reduce hydrogen peroxides and lipid peroxides. However, findings made during the past decade have expanded the functions of selenoproteins beyond redox control. Using the *Gpx1* overproducing and knockout mice, we have unveiled the unanticipated role of this enzyme as a Selenoprotein in regulating insulin gene expression, insulin protein production, insulin secretion after glucose stimulation, and insulin signalling and sensitivity. Subsequently, we have demonstrated impacts and mechanisms of excess dietary Se intakes and altering Gpx1 expression in mice, rats, pigs, and chickens on body glucose, lipid, and protein metabolism. Most recently, we have elucidated that GPX1 inhibited transcription of regenerating islet-derived protein-2 in pancreatic islets, and knockout of selenoprotein V affected responses of functional expressions of four major selenoproteins to dietary selenium and fat intakes in mice. It was intriguing that expression of mRNA or protein of selenoprotein V was undetectable in the liver, but nulling the gene altered hepatic Se concentrations, GPX activities, and mRNA abundances of *Gpx1*, *Selenop*, and *Txnrd1*.

Symposium 3-2
Relative importance of human and mouse selenoproteins

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Mouse has emerged as the most common model organism in biomedicine. Here, we analyzed the tolerance to the loss-of-function (LoF) of selenoprotein genes, estimated from mouse knockouts and the frequency of LoF variants in humans. We found a general correspondence in tolerance (e.g. GPX1, GPX2) and intolerance (TXNRD1, SELENOT) to gene LoF between humans and mice, but also important differences. Notably, humans are intolerant to the loss of iodothyronine deiodinases, whereas their deletion in mice leads to mild phenotypes, and this is consistent with phenotype differences in selenocysteine machinery loss between these species. In contrast, loss of TXNRD2 and GPX4 is lethal in mice, but may be tolerated in humans. We further identified the first human SELENOP variants coding for proteins varying in selenocysteine content. Finally, our analyses suggested that premature termination codons in selenoprotein genes trigger nonsense-mediated decay, but do this inefficiently when UGA codon is gained. Overall, our study highlights differences in the physiological importance of selenoproteins between human and mouse.

Symposium 3-3
The molecular underpinnings of selenium in ferroptosis

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Ferroptosis is emerging as one of the key cell death mechanisms accounting for early cell loss and organ dysfunction in both neurodegenerative disease and tissue ischemia/reperfusion injury (IRI), as occurring during organ transplantation, cardiac infarction and stroke. On the contrary, compelling evidence suggests that therapy-resistant cancer cells and those undergoing epithelial-mesenchymal transition become highly sensitive to ferroptosis, thus representing a still untapped but highly promising Achilles heel for therapeutic intervention based on ferroptosis induction. A first molecular characterization of this death pathway established that the cyst(e)ine/glutathione/glutathione peroxidase 4 (GPX4) axis is at the heart of ferroptosis. The central role of the selenoenzyme GPX4 in ferroptosis regulation is based on its unique function to restrain uncontrolled lipid peroxidation in membranes, thereby preventing the bursting of cells. Genome-wide (CRISPR/Cas9-based) reverse genetic screens identified acyl-CoA synthetase long-chain family member 4 (ACSL4) as an essential downstream player of ferroptosis. In a genetic suppressor screen, we recently uncovered a novel ferroptosis suppressor, called ferroptosis suppressor protein-1 (FSP1, erroneously annotated as apoptosis-inducing factor, mitochondrion-associated 2, AIFM2), which also acts on the level of lipid autoxidation, but independently of the canonical glutathione/GPX4 dependent ferroptosis mechanism. Since this system is expressed in most cancer entities, pharmacological targeting might present a highly attractive strategy to combat difficult to treat tumor entities.

Symposium 3-4
The selenoprotein thioredoxin reductase 1 (TrxR1, *TXNRD1*) as a main regulator of growth factor responses

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The cytosolic selenoprotein TrxR1 is a key enzyme in control of cell function. Its genetic deletion or drug-mediated inhibition typically leads to Nrf2 activation and phenotypic alterations of normal cells, while its inhibition in cancer cells triggers cell death and anticancer effects. One important role of TrxR1 is to regulate growth-factor stimulated protein phosphorylation cascades through modulation of protein tyrosine phosphatases such as PTP1B. This regulatory activity can either be direct or through the action of the TrxR1 substrates thioredoxin (Trx1, *TXN*) and thioredoxin related protein of 14 kDa (TRP14, *TXNDC17*). Regulation of PTP1B activity is multimodal and recent findings reveal that bicarbonate together with hydrogen peroxide (forming peroxymonocarbonate) as well as persulfidation events seem to be important, both of which are regulated by TrxR1.

Symposium 4-1
Role of ferroptosis in carcinogenesis and tumor biology

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Iron is an essential nutrient for all kinds of species on earth. However, iron provides double edged sword; its deficiency causes anemia in mammals but its excess leads to toxic effects. Indeed, excess iron in various pathology has been associated with carcinogenesis. Ferric nitrilotriacetate-induced renal cancers in wild-type rats present similar genetic alterations to their counterparts in humans. Ferroptosis is defined as a form of regulated necrosis, characterized by lipid peroxidation through high iron(oxidants)/sulfur(antioxidants) ratio. Considering that cancer cells generally reserve more catalytic Fe(II) than non-tumorous counterparts, many iron-induced carcinogenesis models, including asbestos-induced mesothelioma, suggest that cancer is a state of “iron addiction with ferroptosis-resistance.” The concept of ferroptosis is now expanding, and ferroptosis is observed not only in pathological conditions (cancer cells after chemotherapy; neurons with aging, etc.) but also in certain physiological conditions. Non-thermal plasma (NTP) is a novel physical technique that emits abundant electrons, resulting in a variety of ROS products by reaction with ambient air. Exposure of NTP to biomolecules, cells or tissues causes oxidative stress *in situ*, leading to DNA breaks and lipid peroxidation products, such as HNE. We found that NTP exposure is highly dependent on Fe(II) *in situ*, causing cancer cell-specific ferroptosis, which was associated with autophagy activation and lysosome genesis. We are trying to visualize ferroptosis in formalin-fixed paraffin-embedded pathology specimens with new monoclonal antibodies, which would reveal physiological function of ferroptosis. Here we discuss the possible role of ferroptosis in cancer research and the use of NTP as a novel cancer

therapy. We also report on our recent observation on the distribution of 8-oxoguanine in the genome and some updates on the issue of asbestos-induced mesothelial carcinogenesis.

Symposium 4-2
Targeting cellular signalling to inhibit tumour cell metastasis and growth: The iron and NDRG1 connection

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The metastasis suppressor, N-Myc downstream-regulated gene-1 (NDRG1) inhibits a plethora of oncogenic signaling pathways by down-regulating the epidermal growth factor receptor (EGFR). Herein, we examined the mechanism involved in NDRG1-mediated EGFR down-regulation. NDRG1 overexpression potently increased the levels of mitogen-inducible gene 6 (MIG6), which inhibits EGFR and facilitates its lysosomal processing and degradation. Conversely, silencing *NDRG1* in multiple human cancer cell types decreased MIG6 expression, demonstrating the regulatory role of NDRG1. Further, NDRG1 overexpression facilitated MIG6-EGFR association in the cytoplasm, possibly explaining the significantly (p <0.001) increased half-life of MIG6 from 1.6 ± 0.2 h under control conditions to 7.9 ± 0.4 h after NDRG1 overexpression. The increased MIG6 levels enhanced EGFR co-localization with the late endosome/lysosomal marker, lysosomal-associated membrane protein 2 (LAMP2). An increase in EGFR levels after *MIG6* silencing was particularly apparent when NDRG1 was overexpressed, suggesting a role for MIG6 in NDRG1-mediated down-regulation of EGFR. Silencing phosphatase and tensin homolog (*PTEN*), which facilitates early to late endosome maturation, decreased MIG6, and also increased EGFR levels in both the presence and absence of NDRG1 overexpression. These results suggest a role for PTEN in regulating MIG6 expression. Anti-tumor drugs of the di-2-pyridylketone thiosemicarbazone class that activate NDRG1 expression also potently increased MIG6 and induced its cytosolic co-localization with NDRG1. This was accompanied by a decrease in activated and total EGFR levels and its redistribution to late endosomes/lysosomes. In conclusion, NDRG1 promotes EGFR down-regulation through the EGFR inhibitor MIG6, which leads to late endosomal/lysosomal processing of EGFR.

Symposium 4-3
Anticancer platinum and gold compounds with thiol-targeting mechanisms of action

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Background: Platinum-based chemotherapy is standard treatment for many types of cancer. The clinically used Pt(II) drugs like cisplatin undergo hydrolysis to form active $[L_2Pt]^{2+}$ species (L = non-labile amine ligands) which crosslinks with DNA and induces DNA damage and apoptosis. However, cisplatin has significant side effects and frequently evokes drug resistance. Herein described are novel anti-cancer Pt(II) complexes which exert their activities via mechanisms distinct from cisplatin and its derivatives by targeting protein thiols of cancer-associated proteins.
Methods: We have prepared cytotoxic Pt(II) complexes with bis-N-heterocyclic carbene (bis-NHC) non-labile chelating ligand and *O*,*O'* leaving group. The stability and reactivity toward biomolecules were determined by LC-MS and fluorescence measurement of the emissive ligand. The cellular localization of the Pt species was traced by using nanoscale secondary ion mass spectrometry (nanoSIMS). A thermal proteome profiling (TPP) analysis was employed to identify cellular target engagement.
Results: The Pt(II) complex (**1a**) displayed significant tumor growth inhibition in mice with higher tolerable doses compared to cisplatin. NanoSIMS imaging of the cellular Pt(II) species showed localization in the cytoplasm but little association with nuclear DNA. The bis-NHC ligated Pt(II) complex formed adducts with cysteine thiols. TPP analysis identified asparagine synthetase (ASNS) as an anti-cancer protein target which contains an active site cysteine residue targetable by the Pt(II) complex. Targeting ASNS limits the availability of asparagine which is needed for supporting cancer cell growth. Accordingly, **1a** treatment reduced cellular asparagine levels and inhibited cancer cell survival which could be partially reversed by asparagine supplementation.
Conclusion: The Pt(II) complex containing bis-NHC ligand displays effective anti-tumor activities. The active bis-NHC ligated Pt species generated from hydrolysis can covalently bind to cysteine residues of anti-cancer protein targets such as ASNS. We have identified a new class of platinum complexes with novel thiol-targeting mechanisms which can be exploited for anti-cancer applications.

Symposium 4-4
Nanochelator of iron for improved iron removal efficacy in various disease models

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Although essential, iron is toxic when in excess and significant pathology accompanies many iron-loading disorders. Deferoxamine (DFO) is an efficient iron chelator with a favourable safety profile, but its onerous parenteral administration limits its clinical use.
We made nanoformulations of DFO using the double emulsion method. *In vivo* iron removal was examined by administering NPs to wild-type mice that had previously been loaded using iron dextran, as well as mouse models of two iron loading disorders, HFE-related hemochromatosis (Hfe-/- mice) and beta-thalassemia intermedia (Hbbth3/+). For brain iron targeting, the NPs were decorated with the RVG29 peptide, and tested in the MPTP murine model of Parkinson's disease (PD).
In wild-type iron dextran loaded mice, and Hbbth3/+ and Hfe-/- mice, DFO-NPs (40 mg/kg DFO; alternate days; 4 weeks) reduced hepatic iron levels, whereas the equivalent values for free DFO were significantly lower. Staining for tissue iron and urinary iron excretion studies confirmed these findings. Pharmacokinetic analysis showed that NP-encapsulated DFO had a much longer elimination half-life than free DFO (48.6 ± 28.8 vs 1.5 ± 0.6 h), and that DFO-NPs could be readily taken up by tissues, and, in particular, by hepatic Kupffer cells. RVG-labelled NPs (35 mg/kg DFO; alternate days; 12 days) were able to deliver DFO to the brain of both wild-type mice and a murine model of PD. They were able to reduce the iron content in the substantia nigra and striatum of these mice and correct the neuro-behavioural defects of the PD animals.
Our results suggest that using a nanoformulation of DFO is a valuable strategy for improving its efficiency as an iron chelator in several different pathological situations, and that this could broaden its clinical use for the treatment of range of human disorders that are characterized by tissue iron loading.

Symposium 5-1
Epigenetics, the third pillar of nitric oxide signalling

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Nitric oxide (NO), the endogenously produced free radical signaling molecule, is generally thought to function via its interactions with heme-containing proteins, such as soluble guanylyl cyclase (sGC), or by the formation of protein adducts containing nitrogen oxide functional groups (i.e. S-nitrosothiols, 3-nitrotyrosine, and dinitrosyliron complexes). These types of interactions result in a multitude of down-stream effects that regulate various functions in physiology and disease. Of the numerous purported NO signaling mechanisms, epigenetic regulation has gained considerable interest in recent years. Our experimental evidence has established that NO is an endogenous epigenetic regulator of gene expression and cell phenotype. We found that cellular exposure to NO modulates the activities of specific epigenetic proteins to regulate gene expression in tumors. These NO-regulated proteins control histone posttranslational modifications (PTM), DNA methylation, and RNA methylation, all of which can significantly affect transcription and/or translation. Specifically, our research determined that NO exposure links modulation of histone PTMs to gene expression changes that promote oncogenesis. Recent studies demonstrated that NO could also significantly alter nucleic acid methylation which controlled the expression of tumor-permissive genes. Additionally, we found that NO can increase chromatin and gene expression heterogeneity, which provide further insight into how NO mediates phenotypic plasticity in tumor cells via epigenetic modulation of chromatin dynamics.

Symposium 5-2
Redox-dependent regulation of chromatin methylation

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The epigenetic landscape describes the chromatin structure of the eukaryotic genome and is a major determinant of cellular transcription. Covalent methylations of both genomic DNA and histone proteins represent critical epigenetic modifications which influence the accessibility of gene loci to the transcriptional machinery. Methylation patterns are determined by the balanced activities of epigenetic writers which add methyl groups and erasers which remove them. While the molecular mechanisms which regulate these processes are not fully understood, evidence from our laboratory suggests that redox-dependent mechanisms are critical.

Methylation depends upon generation and availability of the universal methyl donor, S-adenosyl methionine (SAM), provided by the one-carbon metabolic cycle. Dysregulation of this cycle manifests as pathological changes in plasma levels of the critical metabolic intermediate, homocysteine (Hcy). In turn, elevated plasma Hcy levels correlate with hypo-methylation of DNA. Intriguingly, mutations in the H_2O_2--generating enzyme, NADPH-oxidase-4 (Nox4) were found to associate with altered plasma Hcy levels, and we demonstrated that genetic ablation of Nox4 in mice resulted in significantly lower plasma [Hcy] together with hyper-methylation of DNA within specific tissues via dysregulation of the one-carbon cycle.
Demethylation of DNA is facilitated by successive oxidations of 5-methyl-cystosine (5mC) catalysed by the Ten-Eleven-Translocation enzymes (TETs). TETs are 2-oxoglutarate-dependent dioxygenases and have an absolute requirement for Fe^{2+} which renders their activities susceptible to redox-regulation. Accordingly, we have evidence that the activities of the TET enzymes are inhibited by H_2O_2 generated by Nox4. Accordingly, we found a significant decrease in the abundance of one of the oxidative intermediates of 5mC, 5-hydroxy-methylcytosine (used as a surrogate measure of TET activity) in the hearts of cardiac-specific Nox4 transgenic mice.
We conclude that both the DNA methylation and demethylation processes are regulated by changes in cellular redox, and that Nox4 may be a physiological mediator of these changes *in vivo*.

Symposium 5-3
Regulation of labile Fe(II) and further DNA/histone demethylation by cAMP signaling

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Epigenetic variation reflects the interface of a dynamic environment and the genome. However, it remains elusive how environmental factors influence epigenetic modifications. Cyclic AMP (cAMP) is the second messenger of G-protein coupled receptors (GPCRs) which, as the largest group of membrane receptors, sense environmental cues by binding with ligands. Our lab recently discovered that cAMP in increases the intracellular labile Fe(II) pool likely via Rap guanine nucleotide exchange factor-2 (RapGEF2)-mediated pathway, which is involved in the assembly of vacuolar H^+-ATPase, the H^+ pump responsible for vesicle acidification and labile Fe(II) release to the pool. Labile Fe(II) is an essential cofactor for enzymes, notably, TET methylcytosine dioxygenases and JmjC domain-containing histone demethylases. Addition of Fe(II) to the cell kickstarted TET-mediated DNA demethylation and JmjC-mediated histone demethylation, suggesting that Fe(II) is likely a speed-limiting factor for DNA and histone demethylation. We found that elevation of intracellular cAMP increased 5-hydroxymethylcytosine (5hmC), which can be abolished by iron chelators. Conversely, reduction of intracellular cAMP diminished 5hmC. Intracellular cAMP elevation induced genome-wide 5hmC profile changes which correlate with transcriptome alterations. Furthermore, elevation of intracellular cAMP promoted, while reduction of intracellular cAMP restrained, histone demethylation especially H3K4me3, which also can be abolished by iron chelators. The downregulation of H3K4me3 in response to cAMP is rapid and is correlated with the altered nascent gene transcription. Overall, our studies suggest that cAMP signaling via labile Fe(II) transduces environmental cues into alterations in the epigenome and transcriptome.

Symposium 5-4
Maternal exposure to a mitochondrial toxicant results in life-long alterations in the epigenetic landscape of the offspring

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The crosstalk between mitochondria and epigenetic modifications in the nucleus is becoming increasingly evident with mechanistic links between these events being recently unveiled. Using cell culture models and integrative approaches that relied on biochemical, genetic and 'omics' (RNA-seq, metabolomics and epigenomics) techniques, we previously found that mitochondrial function affects both the DNA methylation and the histone acetylation landscapes through means that go beyond tricarboxylic acid (TCA) cycle intermediates. We also showed that these changes sufficed to influence gene expression and could, to some extent, be reversed pharmacologically or genetically. More recently, we have used an *in vivo* epigenetic biosensor model to interrogate whether inhibition of mitochondrial complex I through the diet of the mother can alter the epigenetic landscape of the offspring later in life. Data obtained on DNA methylation in one locus in the skin and in the whole liver genome will be discussed. Also, results demonstrating how these changes influence gene expression outcomes will be presented.

Symposium 6-1
Relevance and bioactivity of flavonoids as regulators of redox signalling

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In mammals, the bioactivities of flavonoids (as parent compounds or as metabolites) can be mostly ascribed to their chemical and physical interactions with proteins and lipids. This understanding is physiologically relevant at the amounts of flavonoids and of their metabolites bioavailable to most organs and tissues. This is in contrast to unspecific chemical reactions, e.g. free radical scavenging and metal chelation that could barely occur at a significant extent *in vivo*. We have extensively characterized (-)-epicatechin (EC) and EC-related compounds (ECrc) in their capacity to modulate cell redox signaling through extracellular and intracellular actions, and redox and non-redox reactions. Interacting from the outer layer of cell membranes, ECrc can alter lipid rafts, bind to receptors (TLR4) and to functional enzymes (NADPH oxidases) all of which regulates oxidant production. These interactions would be relevant for cells present in certain tissues that are exposed to dietary EC and ECrc, e.g. intestine and vasculature. If incorporated into cells, ECrc can interact with: i) proteins and enzymes that define oxidant levels, mainly NADPH oxidases and NO synthases but also SOD, glutathione peroxidases and catalase; ii) redox sensitive transcription factors and proteins, e.g. NF-κB, Nrf2, and MAPKs; and iii) oxidant species, exclusively relevant at the gastrointestinal tract where ECrc can reach significant concentrations. The actions of EC are consistent with those of other flavonoids, e.g. anthocyanins, in terms of regulating redox-pathways altered in hypertension, diabetes, renal dysfunction, as well as in other pathological conditions. In summary, widespread observations of an *in vivo* antioxidant activity of flavonoids can be explained by their capacity to regulate redox signaling events, rather than direct chemical interactions. Only understanding the molecular mechanisms involved in the bioactivities of flavonoids it will be possible to define which plants and the amounts needed for better promoting health.
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Symposium 6-2
Exercise as an antioxidant supplement to promote healthy ageing and delay frailty

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Exercise is one of the most powerful health-promoting activities that one can undertake. Recently, new European and American guidelines for exercise for promoting a healthy lifestyle are being issued.
From the nutritional viewpoint, a substantial proportion of individuals who perform exercise, take antioxidant supplements.
The major aim of our presentation is to discuss the important role of exercise as a biological antioxidant, because it promotes the expression of antioxidant enzymes and the possible interference with the intake of oral antioxidants, because they blunt the beneficial effects of exercise in terms of promoting antioxidant defence. Some years ago, we reported that oral antioxidant vitamins will blunt the favourable effect of exercise on performance and one year later the group of Michael Ristow proposed that oral antioxidants not only blunt the performance enhancing effects of exercise training, but also the health promoting effects of exercise training and coined the term “mitohormesis”.
Understanding the mechanisms why exercise and nutrition may interfere with each other will be one of the major topics of our presentation. The idea that exercise can be considered as a drug, and moreover as a supplement to treat age-associated frailty, will be discussed in this context.

Symposium 6-3
Ketogenic diets, nutrient signaling and mitochondria

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Calorie restriction increases lifespan in a wide range of animal species. Calorie restriction also induces an increase in fatty acid oxidation and ketogenesis, and it is possible that this shift in metabolism contributes to lifespan extension. The ketogenic diet provides a way to test this idea since it produces a shift in metabolism to fatty acid oxidation and ketogenesis without requiring calorie restriction. Two separate studies have recently shown that ketogenic diets increase healthspan in mice. Ketogenic diets mitigate age-related declines in cognitive and motor function in mice. These diets also preserve lean tissue mass and prevent age-related increases in markers of inflammation. The mechanisms through which ketogenic diets influence longevity and healthspan are not known, although β-hydroxybutyrate is a signaling

molecule that could influence ageing pathways through protein acetylation or interaction with the hydroxycarboxylic acid receptor 2. Ketogenic diets induce a significant increase in protein acetylation that impacts gene transcription (histone acetylation) and may influence the activities of proteins. Inhibitors of the mTOR pathway have been shown to increase lifespan in several species, and ketogenic diets also decrease mTORC1 signaling in some tissues. These diets have been proposed to induce mitochondrial biogenesis and may help maintain mitochondrial content and function with aging. The impact of ketogenic diets on mitochondria, however, is complex and varies by tissue. In particular, these diets prevent decreases in electron transport chain enzyme activities with advanced age in mouse skeletal muscle and brain. Overall, ketogenic diets alter pathways and processes linked with aging and increase healthspan in mice. Development of ketone mimetics may provide a viable strategy to increase healthspan and treat age-related diseases.

Symposium 6-4
Zinc and redox signaling: impact on brain development and function

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The developing brain is highly susceptible to fluctuations in zinc availability. We observed that zinc is critical for neural progenitor cell (NPC) proliferation, migration and differentiation into different neuronal subtypes. Thus, gestational and early postnatal marginal zinc nutrition in rats altered neurogenesis and astrogliogenesis leading to a lower number of neurons and astrocytes, and altered neuronal specification in the brain of the young adult offspring. Accordingly, maternal exposure to the plasticizer DEHP, disrupted zinc homeostasis, decreased zinc supply to the fetus, impairing NPC proliferation and neurogenesis in rats. The adverse effects of zinc deficits on the developing brain are associated to both redox-dependent and –independent mechanisms. In the brain and in neuronal cells, zinc deficits: i) activate NMDA receptors leading to calcium influx and activation of NADPH oxidase and NO synthase, increasing the production of superoxide and NO, ii) alter thiol homeostasis and redox-sensitive signals, iii) cause tubulin oxidation disrupting its polymerization, iv) modulate protein kinases and phosphatases and v) impair ubiquitin-dependent protein degradation. Zinc deficiency ultimately affects the activation of several transcription factors; i.e. Nrf2, NF-κB, NFAT, STAT1/3 and AP-1. Downstream the downregulation of Nrf2, a decreased synthesis of glutathione and other antioxidant defenses affect the capacity of the brain to respond to pro-oxidant stressors when zinc availability decreases. NF-κB, NFAT, STAT1/3 and AP-1 are involved in different aspects of brain development, including NPC proliferation and differentiation into neuronal or glial cell subtypes. Thus, zinc deficits (either because of nutritional deficiencies or secondary to toxicant exposures, infections, chronic diseases) during critical developmental periods can have long-term consequences on brain structure and function. Part of these deleterious effects involve redox-regulated mechanisms.
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Symposium 7-1
Ergothioneine, a thiol/thione antioxidant with therapeutic potential

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Background: Despite the well-established key role of oxidative damage in several human diseases, there has been a general lack of effectiveness of supplements of such “classical” antioxidants as ascorbate, vitamin E and β-carotene in decreasing risk or severity of human disease, and some evidence that high doses over long periods might cause harm. There are multiple reasons for this, one being that these antioxidants are often ineffective in decreasing levels of oxidative damage in humans. They work better in cell culture and in rodent models (which questions the relevance of some rodent models of human disease, and cell culture studies can generate many artefacts).
Methods: The use of biomarkers of oxidative damage in humans has revealed which may be more important antioxidant strategies. We have also used LC/MS to measure ergothioneine in animal tissues and body fluids.
Results: So how then can we minimize oxidative damage in the human body? Effective strategies as revealed by biomarker studies will be discussed. Much of our research now focuses on ergothioneine, a diet-derived antioxidant that is avidly retained by the human body and particularly accumulated at sites of tissue injury, where it may help to diminish tissue damage. We have called it an “adaptive antioxidant” because it seems to concentrate at sites of tissue injury. We have conducted a detailed study of how ergothioneine behaves when administered to humans or mice, and it is showing substantial promise as a potential therapeutic agent in a wide range of human diseases. Data from models of Parkinson disease, Alzheimer disease and other conditions will be presented. Ergothioneine is made only by fungi and some bacteria but is widespread in the human diet.
Conclusion: Ergothioneine may be a very useful diet-derived antioxidant/cytoprotective agent for the treatment and prevention of human diseases.

Symposium 7-2
The emerging role of coenzyme A and protein CoAlation in redox regulation

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Background: Coenzyme A (CoA) is a key metabolic cofactor in all living cells. CoA and its thioester derivatives (acetyl-CoA, malonyl-CoA, HMG-CoA etc.) participate in diverse anabolic and catabolic pathways, allosteric regulatory interactions and the regulation of gene expression. Dysregulation of CoA/CoA derivatives biosynthesis and homeostasis has been associated with various human pathologies, including cancer and neurodegeneration and metabolic disorders.
Methods: To discover and study this novel post-translational modification, termed protein CoAlation, we have developed several novel reagents and methodologies. These include: (a) anti-CoA mAb, which specifically recognize CoA in ELISA, WB, IP and IHC (no anti-CoA antibodies are commercially available); (b) a robust mass spectrometry-based methodology for the identification of CoAlated proteins; and (c) efficient *in vitro* CoAlation and deCoAlation assays. We used a diverse range of biochemical, biophysical, cellular and molecular approaches to explore the role of CoA in redox regulation.
Results: In several published and unpublished studies, we found that protein CoAlation (covalent attachment of CoA to proteins) is a widespread and reversible post-translational modification. It has been detected in single-cell and multicellular organisms, including bacteria, yeast, algae, *C. elegans*, *D. discoideum* and mammals. Cell-based and animal models were employed to demonstrate that protein CoAlation is induced in cellular response to oxidizing agents and metabolic stress. The developed methodology allowed us to identify over 1000 CoAlated proteins in prokaryotic and eukaryotic cells. We showed that protein CoAlation alters the molecular mass, charge, and activity of modified proteins, and protects them from irreversible sulfhydryl overoxidation.
Conclusion: Based on these findings, we propose that under physiological conditions CoA functions to produce metabolically-active derivatives but has a potential to act as an antioxidant in cellular response to oxidative or metabolic stress.

Symposium 7-3
The role of glutathione in bacterial virulence

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Glutathione is important for maintaining intracellular redox homeostasis. Gram-negative bacteria possess the glutathione synthesis genes but they also have transporters to take up exogenous glutathione. In cytosolic pathogens *Burkholderia pseudomallei*, host glutathione regulates bacterial virulence. In *E. coli*, an enteric bacterium that lives in the large intestine where glutathione concentration is high, glutathione can be taken up and metabolized to hydrogen sulphide, which has some consequences for the host.
I will present these two examples of how an intracellular pathogen and extracellular commensal or opportunistic pathogen make use of host glutathione for their survival. Through bacterial genetics, cell biological and biochemical techniques, we found that upon escape from the oxidizing environment of the phagosome into the host cytosol, *B. pseudomallei* makes use of host glutathione to activate the membrane-bound histidine kinase sensor VirA that leads to activation of its cognate DNA response regulator, which in turn activates bacterial virulence. Conversely in *E. coli*, we serendipitously found that in the nematode *C. elegans*, a surrogate model of bacterial virulence, *E. coli* and glutathione exerts rapid killing on *C. elegans* which had been synchronized in their life cycles with 5 fluorodeoxyuridine. The rapid killing is mediated by hydrogen sulphide that is produced by the bacteria and acts together with 5 fluorodeoxyuridine to mediate death.
In one instance, host glutathione acts as a spacio-temporal cue for an intracellular pathogen to switch on its virulence program at the right time and place. In the other example of enteric bacteria encountering glutathione in the gut, they have the enzymes to convert exogenous glutathione to hydrogen sulphide. This could lead to host cell death in the context of colorectal cancer when patients receive 5-fluorouracil as an anti-cancer drug. As host glutathione is ubiquitous, different bacteria have evolved mechanisms to fully exploit it for their own purposes.

Symposium 7-4
Human peroxiredoxin 3: oxidizing substrate specificity, glutathionylation and other oxidative post-translational modifications

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Peroxiredoxin 3 is a thiol-dependent peroxidase expressed in mitochondria, where it plays a major role in hydrogen peroxide reduction. We have recently reported that peroxiredoxin 3 also catalyzes the reduction of peroxynitrite, formed from the diffusioncontrolled reaction between superoxide and nitric oxide radicals. Kinetic data indicate both peroxiredoxin 3 and 5 as main targets for mitochondrial peroxynitrite. A minor fraction of the latter is expected to react with mitochondrial CO2 and to form carbonate and nitrogen dioxide radicals, which in turn participate in further oxidation and nitration reactions. Additionally, we found that peroxiredoxin 3 reduces fatty acid hydroperoxides, which can be formed either non-enzymatically or enzymatically inside the mitochondria. The rate constants so far determined indicate that the enzyme has a broad oxidizing substrate specificity, catalyzing the reduction of these different substrates with $k \geq 107$ M-1s-1 at pH 7.8 and 25 °C. In addition to oxidizing the so called peroxidatic cysteine residue (CysP) from thiol to sulfenic acid, in the oxidative step of the catalytic cycle, these oxidants caused the inactivation of the enzyme due to hyperoxidation of CysP from sulfenic acid to sulfinic acid. This reaction competes with the disulfide formation with the resolving cysteine residue (CysR) from another subunit in the dodecameric enzyme (resolution step of catalysis). Furthermore, the sulfenic acid at CysP reacted with GSH with a rate constant of 400 M-1s-1 which, considering the high mitochondrial GSH concentration indicates that enzyme glutathionylation should compete with resolution in the organelle. Glutaredoxin 2, expressed in the mitochondria, reduced glutathionylated peroxiredoxin 3. Thus, GSH/Grx2 provides an alternative route of peroxiredoxin 3 reduction, while preventing enzymatic inactivation due to hyperoxidation.
In conclusion, peroxiredoxin 3 possess multiple oxidizing as well as reducing pathways in mitochondria, and can be subject of different oxidative post-translational modifications with impact in protein function

Symposium 8-1
NRF2 interacts with STAT3 and induces IL-23 expression in breast cancer

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Background: Nuclear factor E2-related factor 2 (Nrf2) is a redox-sensitive transcription factor regulating the expression of a battery of genes encoding majority of antioxidant enzymes. The cellular stress response or cytoprotective signaling mediated by Nrf2 is often hijacked by cancer cells. This may facilitate the remodeling of the tumor microenvironment making it advantageous for the autonomic growth of cancer cells, metastasis, angiogenesis, and tolerance to anticancer therapy. A subpopulation of cancer cells, termed cancer stem cells (CSCs), has stemness properties, such as self-renewal and differentiation, which drive cancer recurrence and tumor resistance. CSCs possess enhanced protection capabilities to maintain reduced intracellular levels of reactive oxygen species (ROS) compared to non-stem-like cancer cells. This study was aimed to investigate whether Nrf2-mediated reductive stress could regulate self-renewal activity in breast CSCs.
Methods: In this study, we investigated the involvement of Nrf2 in human breast cancer stem-like cells. The expression of Nrf2 and its target genes and proteins were measured by RT-PCR and Western blot analyses. Breast cancer stem-like cells were sorted by flowcytometry. The in vivo tumor growth was assessed in a xenograft mouse model. The expression of stem cell marker proteins and related signaling molecules in the tumor tissues was determined by immunohistochemical analysis.
Results: We found that manifestation of stemness in breast cancer stem-like cells was associated with an elevated production of reduced glutathione (GSH) maintained by upregulation of glutamate cysteine ligase catalytic subunit (GCLC) and consequently, lowered ROS levels. This was accompanied by upregulation of P-AMPK, FoxO3a and Bmi-1. Notably, expression of Nrf2 protein was substantially increased in cells undergoing sphere formation. We noticed that expression of FoxO3a was inhibited following introduction of Nrf2 siRNA into MCF-7 mammosphere cells. Silencing of Nrf2 expression suppressed the xenograft growth of subcutaneously or orthotopically injected human breast cancer cells.
Conclusion: Nrf2 overactivation in breast CSCs, which, in turn, upregulate GCLC expression and consequently enhances GSH biosynthesis with concurrent reduction in intracellular ROS accumulation, provoking the reductive stress. The consequent upregulation of nuclear FoxO3a and its binding to the Bmi-1 promoter, may account for the self-renewal activity of breast cancer stem-like cells and their growth in a xenograft mouse model.
Keywords: Nrf2, multi-stage carcinogenesis, heme oxygenase-1, cancer stem cells

Symposium 8-2
Cytoprotective function of NRF2 and its role in sulfur metabolism

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Background: Keap1-NRF2 system is a sulfur-based cytoprotection mechanism. KEAP1 utilizes multiple cysteine residues for sensing electrophiles and reactive oxygen species, whereas NRF2 regulates various genes whose products catalyze redox reactions in which sulfur atoms play important roles. NRF2 activation is beneficial for human health by increasing anti-oxidant and detoxification capacities. Our recent work revealed that NRF2 has also a potent anti-inflammatory activity. In addition, several papers have described that NRF2 enhances mitochondrial activity. However, a precise mechanism how NRF2 enhances the mitochondrial activity has not been fully understood.
Methods: Based on our recent discovery in collaboration with Prof. Takaaki Akaike at Tohoku University Graduate School of Medicine, that mitochondrial sulfur metabolism makes a substantial contribution to generation of the mitochondrial membrane potential, we hypothesized that NRF2 promotes the mitochondrial activity through increasing supply of cysteine as a substrate. We examined contributions of NRF2 and its downstream effectors to the generation of the membrane potential by knocking down each factor in cultured cells.
Results: NRF2 knockdown and KEAP1 knockdown decreased and increased the mitochondrial membrane potential, respectively, verifying the NRF2 role in the regulation of the membrane potential. Depletion of cystine from culture medium, knocking down one of the NRF2 target genes, *SLC7A11* encoding a cystine transporter xCT, and pharmacological inhibition of xCT by sulfasalazine all dramatically decreased the mitochondrial membrane potential, indicating the requirement of cysteine for the mitochondrial activity. Knockdown of sulfur-metabolizing enzymes in mitochondria all decreased the membrane potential, indicating an essential contribution of the sulfur metabolism to the mitochondrial activity.
Conclusion: With these results, we concluded that NRF2 enhances mitochondrial activity by elevating SLC7A11 expression, leading to the increased supply of cysteine and promotion of mitochondrial sulfur metabolism for generation of mitochondrial membrane potential.

Symposium 8-3
Targeting Keap1/Nrf2 as a strategy to break the vicious cycle of oxidative stress – inflammation – cell death

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Disrupted redox and protein homeostasis and chronic inflammation engage in a vicious cycle that leads to cell death, and are underlying the pathogenesis of many chronic diseases. Transcription factor nuclear factor erythroid 2 p45-related factor 2 (Nrf2) and its binding partner Kelch-like ECH-associated protein 1 (Keap1), regulate the expression of large networks of genes encoding proteins that provide powerful and long-lasting protection against damage by oxidants and pro-inflammatory agents. Many pharmacological activators of Nrf2 (termed inducers) are electrophiles that reversibly bind to highly reactive sensor cysteines within Keap1. Non-electrophilic inducers that disrupt the Keap1-Nrf2 protein-protein interactions by binding to the Kelch domain of Keap1 are also emerging. In both cases, the ability of Keap1 to target Nrf2 for degradation is impaired, allowing for Nrf2 accumulation and enhanced transcription of Nrf2-dependent genes. The Keap1/Nrf2 regulatory network includes drug metabolizing, antioxidant, anti-inflammatory, metabolic enzymes, as well as autophagy-related proteins and thus Nrf2 has a critical role in the maintenance of the cellular redox and protein homeostasis, and in the resolution of inflammation. In addition, Keap1 downregulation confers features of a fasted metabolic state, and provides protection against metabolic stress. Inducers of Nrf2 that inactivate Keap1 are protective in multiple cell culture and animal models of chronic disease and have documented beneficial effects in humans.

Symposium 8-4
ROS signalling and Nrf2-mediated adaptive response in type 2 diabetes

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Background: The development of type 2 diabetes (T2D) is a result of progressive worsening of insulin responsiveness and loss of functional pancreatic β-cells. Amidst the various pathophysiological mechanisms proposed, oxidative stress is a common denominator. However, the key factors that link oxidative stress to insulin resistance and/or β-cell dysfunction have not been clearly defined. Nuclear factor E2-related factor 2 (Nrf2) is a CNC-bZIP transcription factor that is well established as a master regulator in the cellular adaptive response to oxidative stress.
Methods: We have tested our hypotheses in a variety of cell, mouse and computational models and demonstrated that Nrf2 and adaptive antioxidant response play paradoxical roles in regulating the function of β-cells and various insulin-responsive cells, by which affect glucose and lipid homeostasis.

Results: Our studies demonstrated that reactive oxygen species (ROS) may function as important intracellular signalling molecules to mediate glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells and insulin action in insulin responsive cells. The magnitude of ROS signalling is inversely correlated with the ROS-scavenging activity and antioxidant status in cells. When cells are chronically exposed to oxidative stressor(s), cellular ROS-scavenging capacity is adaptively upregulated, mainly through activation of Nrf2 and subsequent transcriptional induction of a suite of antioxidant enzymes. The induced antioxidant enzymes, meant to maintain intracellular redox homeostasis and limit oxidative damage, may produce an undesired effect by impeding ROS that function as physiological signalling molecules. Conclusion: We propose that Nrf2-mediated antioxidant response plays paradoxical roles in β-cell function and insulin action: (1) It protects the cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment in insulin secretion and action; (2) Since ROS signalling triggered by glucose or insulin could be an important component involved in insulin secretion and action, the induction of endogenous antioxidants in the presence of oxidative stress may blunt the signals, resulting in reduced GSIS, insulin resistance and T2D.
Keywords: ROS signalling, NRF2, type 2 diabetes

Symposium 9-1
Regulation of neurovascular coupling in the brain mediated by nitric oxide: the redox cycle of ascorbate and nitrite

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Neurovascular coupling (NVC) orchestrates the rapid and transient delivery of bioenergetic substrates by the local vasculature to neighboring cells, according to energy demands imposed by neural activation. Failure in neurovascular coupling, either during aging and disease (Alzheimer´s disease, AD) or following acute hypoxic conditions, compromises brain integrity and functionality. The regulation of NVC is under the concerted cooperation of the cells comprising the neurovascular unit and recently, by demonstrating quantitatively and *in vivo* a spatial, temporal, and amplitude association between cerebral blood flow (CBF) changes, O_2 tension and NO dynamics, we have supported the notion that NO derived from the neuronal nitric oxide synthase isoform (nNOS) acts as a direct mediator of NVC in hippocampus. We have come to conjecture that, under conditions in which nNOS is not fully operative, including hypoxic conditions, the redox and functional interplay of ascorbate and nitrite would modulate the functionality of NVC via NO production. By using a multimodal approach to probe the dynamics of NO, ascorbate and CBF *in vivo* in hippocampus of rodent models, we support that (1) neuronal-derived NO acts as a direct mediator of neurovascular coupling, (2) volume signaling by NO is an intrinsically controlled mechanism due to increased blood flow, (3) neurovascular coupling is impaired in AD and aging in terms of both amplitude and delay of vascular responses to NO signal and (4) the redox interaction of nitrite and ascorbate in hippocampus increases NO bioavailability, augments CBF and improves cognition. Thus, one may envisage that the redox cycle of ascorbate and nitrite in hippocampus might mechanistically support a dietary approach to sustain neurovascular coupling, attenuating cognition decline in aging and disease.
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Symposium 9-2
Calcium-dependent mechanisms of cerebral blood flow regulation

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Brain's electrical activity correlates strongly to changes in cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen ($CMRO_2$). Brain oxygen consumption is correlated and controlled by Na,K-ATPase activity driven by transmembrane currents, while Ca^{2+}-signals control activity-dependent rises in CBF. In the past, brain capillaries were viewed as tubes that passively conducted blood from the heart to the active nerve cells. However, recent data provided by others and by us suggest that brain capillaries may have control mechanisms for blood flow regulation, and novel microscopy techniques have now made it possible to examine the blood-brain barrier (BBB) in more detail in living animals as well. I report that rises in synaptic activity in mouse somatosensory cortex evoke capillary dilation that mostly starts in capillaries before arterioles. The capillary dilation is initiated at precapillary sphincters and 1st or 2nd order capillary, from where it propagates upstream to the penetrating arteriole and downstream to higher order capillaries. Application of the gliotransmitter ATP induces dilation followed by constriction that also propagates up- and downstream at velocities of 5-20 µm/s, i.e. at a similar speed as an intracellular calcium wave. In pathology, e.g., cortical spreading depolarization (CSD), which is thought to trigger migraine in patients, the sphincter and capillaries contract, decreasing the lumen and increasing the length of the segment during the period of low blood flow that concurs with migraine symptoms. In normal function and CSD the fluctuations of vascular diameters are contra-correlated to cytosolic Ca^{2+} in

vascular smooth muscle and bran pericytes. In the case of cardiac arrest, precapillary sphincters and capillaries demonstrate a total collapse ~10-20 min after onset of ischemia. This timing is similar to the timing of the so-called 'no-reflow' phenomenon in global ischemia. Our study provides unique insights into the correlation between Ca^{2+} rises in nerve cells and vascular cells as key to an understanding of brain vascular control.

Symposium 9-3
Calcium-dependent mechanisms of cerebral blood flow regulation

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Ischemic stroke is the second most common cause of death worldwide, yet clinical treatment with Alteplase is limited by its narrow therapeutic window of 6h. Notably, studies examining protection afforded by plant-derived compounds *in vitro* or rodent models of ischemic stroke have failed to translate clinically. We previously reported that the dietary isothiocyanate sulforaphane (SFN), known to activate phase II and endogenous antioxidant enzymes via the Keap1-Nrf2 defense pathway, affords neurovascular protection and improves behavioral outcomes following cerebral ischemia-reperfusion injury (Alfieri et al., FRBM 2013; 65:1012-1022). We recently investigated redox signaling in brain microvascular endothelial cells (bEnd.3) exposed to ischemia-reoxygenation as an *in vitro* model of ischemic stroke.bEnd.3 cells adapted long-term (5d) in a Scitive workstation to atmospheric (18 kPa), physiological (5 kPa) and hypoxic (1 kPa) O_2, noting that standard culture under 18 kPa O_2 exposes cells to hyperoxia and oxidative stress. A nanoparticle phosphorescence probe (MitoXpress-Intra) confirmed that cells adapted to 5 kPa O_2 recapitulate intracellular O_2 levels (3.6 kPa) reported *in vivo*. Under 18 kPa O_2 bEnd.3 GSH levels were significantly higher than in cells under 5 kPa, confirming our findings in lung epithelial cells (Kumar et al., FEBS Letters 2016, 590:258-69). Notably, adapting cells to 5 kPa O_2 resulted in reduced expression of Nrf2 target proteins (HO-1 and GCLM) in response to SFN, consistent with our findings in umbilical endothelial cells (Chapple et al., 2016, FRBM 92:152-62). When bEnd.3 cells were subjected to acute hypoxia-reoxygenation to simulate reperfusion injury, re-oxygenation led to a rapid burst of reactive oxygen species which was significantly reduced by pretreatment with PEG-SOD or pretreatment with SFN (2.5 μM). This lecture aims to highlight the critical importance of conducting *in vitro* experiments under carefully regulated and physiologically relevant oxygen levels (Keeley & Mann, Physiol. Reviews 2019; 99:161-234).
Supported by BHF and HRUK

Symposium 9-4
Glutamate-glutamine cycling and the oxidative metabolism rate in astrocytes

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Astrocytes play an important role in glutamatergic neurotransmission, namely by clearing synaptic glutamate and converting it into glutamine that is transferred back to neurons. The rate of this glutamate-glutamine cycle is known to couple to that of glucose utilization and of neuronal metabolism. While neurons have high oxidative capacity, astrocytes are often considered to be glycolytic cells with meagre mitochondrial oxidative metabolism. Magnetic resonance spectroscopy has been used for ^{13}C tracing experiments *in vivo*, namely for detecting labelling incorporation from ^{13}C-labeled glucose into brain metabolites and major amino acids. Such approach allows to determine rates of energy metabolism in neurons and astrocytes, and the glutamate-glutamine cycle. Our recent work in the cerebral cortex of animal models suggests that variations of the glutamate-glutamine cycle rate upon cortical stimulation are coupled to the rates of mitochondrial metabolism in both neurons and astrocytes. Moreover, while the rate of resting energy metabolism is slower in astrocytes than neurons of the cortex *in vivo*, somatosensory stimulation induces oxidative metabolism increments of similar magnitude in the two cell types. Finally, we have investigated brain energy metabolism changes in insulin resistant Goto-Kakizaki rats and found an imbalance between neuronal and astroglial oxidative metabolism that results in impaired glutamate-glutamine cycle. These results are in line with diabetes-induced astrogliosis and disrupted astrocytic support to synaptic activity.
Altogether, these results are in line with an active role of astrocyte bioenergetics in glutamatergic neurotransmission, which is key in disorders characterised by dysfunction of excitatory synapses.

Symposium 10-1
Sirtuins and mTOR in aging pathways – the role of cell senescence

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There is a growing sense that a holistic understanding of ageing biology may be achievable. This would represent a tremendous advance in our collective biological understanding and afford opportunities for novel interventions to enhance human healthspan. Ageing is the biggest risk factor for the major chronic diseases growing in prominence. These include cardiovascular and neurodegenerative diseases, diabetes and cancer. If ageing can be slowed, the effect would be simultaneous protection from many of the chronic diseases. One strategy is to use animal model organisms to find common pathways that modulate ageing and then to seek methods for their manipulation in humans. The TOR pathway is one point of convergence and a clinically approved drug targeting the TOR kinase, rapamycin, extends murine lifespan and healthspan. Sirtuin deacteylases represent another set of conserved aging regulators. Both pathways have been linked to DNA damage and cellular senescence. Understanding the mechanisms by which mTOR and Sirtuins regulate aging remains a critical goal of aging research. Here I will discuss links between these regulators and known aging pathways, and the possibility to develop interventions that extend human lifespan and healthspan.

Symposium 10-2
Repurposing approved drugs as geroprotectors: Experimental versus epidemiological evidence

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Due to a rapidly aging society, pharmacological interventions to delay the onset of age-associated diseases have been identified to be of utmost relevance. Repurposing previously approved drugs and compounds are advantageous given their cost-effectiveness and safety profile. The latter is based on long-standing epidemiological evidence regarding the ideal case, absence of side effects. Likewise, these pre-existing data allow to estimate whether such compounds are capable of affecting overall and disease-specific mortality. The presentation will compare such and in wider parts redox-related drugs, including metformin, EGCG, glucosamine and lithium salts, regarding their respective risks, costs and benefits with regards to the prevention and amelioration of chronic diseases of the elderly.

Symposium 10-3
Mitochondrial Metabolism in T Cell Activation and Aging

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Aging of the immune system is characterized by loss of adaptive immunity and increase in non-specific innate immunity. Amongst the immune system components most affected by aging are T lymphocytes, which play a central role in the immune defense against intruders, as well as maintaining tissue homeostasis and function. T cell stimulation and effector functions are metabolically demanding, and the ability to reshape intracellular metabolism is crucial for T cells to exit quiescence. Our studies demonstrate that the metabolic environment of the organ from which aged T cells are collected affects their metabolic fitness and ability to activate. Failure to activate is connected to skewed metabolic reprogramming, whereas metabolite supplementation rescues aged T cells response to stimulation. Our studies suggest that both systemic and cellular changes in metabolism contribute to reduced T cell immunity in the aged.

Symposium 10-4
Exercise and vascular ageing: endothelial redox regulation by Sirt1

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The chronic inflammation of vascular cells has been shown to cause atherosclerosis. Pathological properties, including endothelial cellular dysfunction, the migration of lipid particles, foam cell generation and the migration of macrophages,

have been identified in atherosclerotic lesions. HHcy (hyperhomocysteinemia) is one of the main risk factors for cardiovascular diseases by increasing the incidence rate of atherosclerosis. In addition, SIRT1, is a principal protein for maintaining the health of the human cardiovascular system. SIRT1 acts as a cytoprotective regulator that protects the cardiovascular system from degeneration and oxidative injuries. Our study demonstrated that the Hcy-induced LOX-1 up-regulation through an elaborate pathway relates to the PKCβ up-regulation and NADPH oxidase activation. We also confirmed that HSF1 acetylation was enriched by the Hcy-induced degradation of SIRT1, thereby mitigating its function to attenuate Hcy-induced LOX-1 up-regulation. Moreover, the PKCβ inhibition and SIRT1/HSF1 overexpression protected the endothelial cells from Hcy-induced apoptosis. The previous study indicated that exercise training enhances SIRT1 activity in aged animals and increases antioxidant capacity. Our group revealed that exercise training significantly reversed SIRT1 inhibition as well as pro-apoptotic and pro-inflammatory events in animals receiving methionine intervention (HHcy animals). All findings from the present study suggested that exercise training might be a method for interrupting endothelial apoptosis and preventing the development of atherosclerosis through SIRT1 activation and oxidative stress inhibition under HHcy conditions. On the other hand, the role of SIRT1 in coronary artery disease (CAD) was investigated in our group. We found that the SIRT1 expression levels were repressed and the acetylated p53 expression levels were enhanced in the monocytes of patients with CAD. LOX-1/oxidative stress was also up-regulated in the monocytes of patients with CAD, thereby increasing pro-apoptotic events and pro-inflammatory responses. Results from our study might provide new knowledge with respect to the management of clinical CAD patients.

Symposium 11-1
Molecular regulation of mitochondrial autophagy and cellular fate

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Mitochondria are highly dynamic organelles and respond to stress by changing their fission-fusion cycle, undergoing mitophagy, or releasing apoptotic proteins to initiate cell death. Mitophagy is a selective process that removes damaged or unwanted mitochondria. We have previously revealed that FUNDC1, a mitochondrial outer-membrane protein, functions as a mitophagy receptor to mediate hypoxia-induced mitophagy. FUNDC1 harbors an LC-3–interacting region (LIR) and interacts with LC-3 to mediate mitophagy. PGAM5, a mitochondrially localized phosphatase, is able to dephosphorylate FUNDC1 to promote mitophagy. Interestingly, we found that Bcl-xL interacts with PGAM5 to inhibit its phosphatase activity and the subsequent activation of mitophagy. We further showed that PGAM5, which exists in an equilibrium between dimeric and multimeric states in response to mitochondrial oxidative stress, dephosphorylates Bcl-xL to inhibit apoptosis. We suggest that the reciprocal interaction of PGAM5 with FUNDC1 and Bcl-xL serves as a molecular switch between mitochondrial fission/mitophagy and apoptosis.

Symposium 11-2
Oxidative stress induced mitophagy in the aging heart

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Background: Aging is a major risk factor for developing heart disease. There is increased oxidative stress in the aged heart that causes mitochondrial damage. Autophagy is a cellular degradation pathway involved in eliminating dysfunctional mitochondria from cells. Here, we have investigated how autophagy and mitophagy are altered with age in the heart.
Methods: We evaluated cardiac structure and function in young (4 mo) and aged (24 mo) mice by echocardiography and histology. We also evaluated autophagy and mitophagy by analysing mRNA and protein level of autophagy regulators by qPCR and Western blot analysis, respectively. Hearts were also evaluated at the ultrastructural level by transmission electron microscopy (TEM).
Results: We found that aging was associated with increased cardiac fibrosis, hypertrophy and inflammation, as well as diastolic dysfunction. We also found that autophagic flux was reduced in the aged hearts and that this was due to reduced formation of autophagosomes. A qPCR array analysis comparing transcript levels of key autophagy regulators in young and aged hearts revealed a significant decrease in several Atg proteins, confirming reduced formation of autophagosomes in aged heart. The reduced autophagosome formation correlated with increased accumulation of ubiquitinated cargo in the aged heart. We also found that the E3 Ubiquitin ligase Parkin, a key mitophagy regulator, was significantly increased in the aged hearts. Analysis of mitochondrial fractions demonstrated increased levels of Parkin, ubiquitinated proteins and p62, an indication that they have been labelled for mitophagy. Finally, TEM showed accumulation of elongated mitochondria in the aged heart.

Conclusion: There is an imbalance in the labelling and the degradation steps in the aged heart which leads to accumulation of ubiquitinated mitochondria. Failure to remove these mitochondria likely leads to their fusion with healthy mitochondria as an attempt to dilute damage.
Keywords: Mitochondria, mitophagy, autophagy, aging, heart

Symposium 11-3
The novel function of mitochondrial outer membrane protein Fis1 in mitochondrial dynamics and quality control

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Background: Adapting to a variety of physiological demands, mitochondria converge stresses and respond to maintain cellular homeostasis, fine-tuning a plethora of cellular processes. Despite little is known about the lateral organization and structure of mitochondrial outer membrane, outer mitochondrial membrane (OMM) proteins are highly emphasized in mitochondrial dynamics, coupled with continuous fission and fusion, to coordinate various mitochondria-associated processes, including mitophagy/autophagy and apoptosis. Recently, our group focuses on deciphering the functions of two OMM proteins, Fis1, which was originally thought to have important functions in mitochondrial fission machinery; however, their functions on mitochondrial quality control have not yet been well-elucidated.
Methods: With imaging approaches including structure-illumination microscopy (SR-SIM), here we demonstrate that the SNARE protein Syntaxin 17 (STX17), initiates mitophagy upon the depletion of mitochondrial outer membrane protein Fis1. Using proteomics analysis, we identify STX17 as a novel interacting partner for Fis1, which preferentially governs the dynamic shuffling of STX17 between ER and mitochondria. Loss of Fis1 results in the aberrant accumulation of STX17 on mitochondria and mitochondria-associated membranes (MAM), exposing its N terminus to assemble and self-oligomerize for mitophagy. Mitochondrial STX17 interacts with ATG14 and further recruits core autophagy proteins hierarchically, to form mitophagosomes, followed by Rab7-dependent mitophagosome-lysosome fusion.
Results: Our results reveal that Fis1 loss impairs mitochondrial respiratory function, and potentially sensitizes mitochondria to STX17-mediated mitochondrial engulfment within autophagosomes, which is directly initiated through canonical autophagy machinery, closely linking non-selective macroautophagy and mitochondrial removal.
Conclusion: Our findings uncover a novel PINK1/Parkin-independent mitophagy mechanism, in which mitochondrial outer membrane protein Fis1 gatekeeps the clearance of mitochondria.

Symposium 11-4
Autophagy of mitochondria and their association with the nucleus in mammals

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Mitochondria are central to the homeostasis of mammalian cells in which they play part beyond the production of energy. By regulating both intracellular signalling and programmed cell death they are critical in the onset and progression of diseases. Surprisingly though, their interaction with the surrounding environment, following failure of mitochondrial autophagy, remains under investigated limiting our capacity to detail conduits for pharmacological targeting and design of innovative therapies in chronic conditions characterized by underlying redox-stress. In my talk I shall overview the research we are pursuing to inform the biology and pharmacology mitochondria highlighting our ongoing work on the physical interaction between mitochondria and nucleus. The molecules involved in this route of communication will be detailed together with the approaches devised to tackle and revert this mechanism of cellular maladaptation and chemotherapy failure. Finally, I shall illustrate how the formation of the contact sites between mitochondria and nucleus referred to as NAM (Nucleus Associated Mitochondria) redistribute cholesterol inhibiting the SIRT-1 mediated deacetylation of intranuclear proteins including LC3 and thereby repressing general and targeted autophagy (mitophagy).
Key words: Mitochondria, Pharmacology, Cancer, NAMand Mitophagy
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Symposium 12-1
Peroxiporins in subcellular redox homeostasis and signaling

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Background: H_2O_2 is a key second messenger that potentiates tyrosine phosphorylation circuits inhibiting phosphatases and activating a few kinases. A key question is how H_2O_2 signals can reach their targets in the molecularly crowded cytosol. We showed that efficient H_2O_2 transport across biological membranes requires aquaporin 8 (AQP8) or other peroxiporins. Transport via AQP8 is gated by persulfidation of a conserved cysteine (C53), which modulates signal intensity and duration. What is the role of other AQP family members in intercellular and interorganellar redox signaling?
Methods: We expressed suitably tagged AQP11 variants in human cells and investigated their localization, activity and regulation by imaging and biochemical assays.
Results: Unlike AQP8, AQP11 does not reach the cell surface but exerts its peroxiporin activity in the endoplasmic reticulum /ER) membrane. Part of it is present in mitochondrial associated ER membranes. Silencing or overexpressing it impacts the basal H_2O_2 levels in the ER lumen. Drugs that induce ER stress gate AQP11 peroxiporin activity by redox sensitive mechanisms.
Conclusion: AQP11 is a peroxiporin uniquely localized in the ER. It allows and regulates H_2O_2 fluxes across the ER membrane. Considering that disulfide bond production by the Ero1 pathway entails H_2O_2 production and NOX4 is also an ER resident enzyme, the permeability of AQP11 may control key interorganellar signals. Its restricted topology may be important for localizing redox signals in restrained areas of the cytosol, particularly in conditions of ER stress.
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Symposium 12-2
Cellular hydrogen peroxide nanodomains

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Background: Physically tethered contacts between mitochondria and ER enclose a nanometer-scale interface that responds dynamically to stimuli including metabolic and disease states. At these contacts, local Ca^{2+} delivery from ER to the mitochondria induces H_2O_2 nanodomains, which are required to maintain Ca^{2+} signalling. However, it remains elusive whether single mitochondria can also initiate local reactive oxygen species (ROS) communication to affect their environment.
Methods: To record single mitochondrial and ER-mitochondrial contact activities fluorophores and organelle-targeted ROS and Ca^{2+} sensing fluorescent protein constructs were used in combination with live microscopy in HepG2 and genetically modified HEK cells.
Results: We show that single mitochondrial transient depolarization events (flickers) induce oxidative bursts confined to the close vicinity of the mitochondrion, which are sensed by the adjacent IP_3 receptor Ca^{2+} channels of the ER. Flickers and oxidative bursts occur in the absence of any perturbations but become more frequent in cells under mitochondrial stress caused by oligomycin. Furthermore, during stress caused by a pro-apoptotic agent, staurosporine, increased mitochondrial flickering involves an IP_3 receptor-mediated Ca^{2+} release and mitochondrial Ca^{2+} uptake positive feedback mechanism that is central to the execution of the cells.
Conclusion: Mitochondria are engaged in periodic membrane potential resetting and ensuing oxidative bursts confined to the neighboring organelles. The localized oxidative bursts modulate physiological signalling mechanisms and under stress conditions, might support recycling of the impaired mitochondria or the whole cell.

Symposium 12-3
Estimating compartmental hydrogen peroxide steady-state concentrations using experiment and theory

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Background: Steady-state concentrations of hydrogen peroxide in different cellular compartments are of interest for a variety of reasons. The detailed mechanisms of most H_2O_2-mediated signalling reactions, including those that control cell fate, are currently unknown. Knowledge of relevant concentration ranges can inform experimental design. Similarly, quantitative differences in hydrogen peroxide metabolism in normal versus disease states are currently unknown.

Methods: Genetically-encoded sensing and perturbation tools, redox Western blots, ^{13}C isotopic tracers, phenotypic assays, and kinetic models parametrized with experimentally determined protein abundances and rate coefficients were used to quantify basal and toxic H_2O_2 steady states in the mitochondria and cytosol of HeLa cells.
Results: Steady-state basal concentrations of H_2O_2 are in the tens of picomolar range in the cytosol and single-digit nanomolar in the mitochondria. Upon increased generation of H_2O_2 in either compartment, apoptosis occurs prior to the collapse of the cytosolic and mitochondrial peroxiredoxin pools. The cytosol is more robust to H_2O_2 perturbations than the mitochondria.
Conclusion: With methodology established, it is now of interest to analyse and compare a variety of cell and tissue types, normal and pathological. Examination of additional compartments may require advances in measurement tools. Many experiments aimed at uncovering H_2O_2-mediated signalling mechanisms are performed using excessive H_2O_2, which may lead to non-physiological oxidation reactions that obscure the relevant ones.
Keywords: Hydrogen peroxide; peroxiredoxin; cytosol; mitochondria; genetically-encoded tools; mathematical model

Symposium 12-4
Modeling of cellular hydrogen peroxide landscape

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Background: Hydrogen peroxide (H_2O_2) regulates signaling pathways by modulating the activity of redox sensitive proteins denominated redox switches. The magnitude of the transient variations in localized H_2O_2 pools during signaling events and how these variations impact redox switches present in the cell remain elusive. Here, quantitative biology concepts are applied to probe the cellular hydrogen peroxide signaling landscape.
Methods: A canonical model with two chemical reactions comprising the oxidation/reduction cycle of a redox switch was set up. The model is dimensionless with respect to the redox switch concentration, so that the experimental data required to apply the equations deduced is the percentage of oxidation of a redox switch. This avoids application of absolute concentrations, which are difficult to measure experimentally.
Results: The canonical model predicts a simple principle for H_2O_2 signaling: the magnitude of the response of a redox switch to H_2O_2 depends on the ratio between the rate constants that describe the reduction and the oxidation of the redox switch, while the response time depends on the sum of these processes. Based on this principle, it was predicted that PTP1B oxidation by H_2O_2 *in vivo* is mediated by peroxymonocarbonate, which was recently confirmed experimentally. The model was also used as an analytical tool to probe the absolute concentrations and gradients of H_2O_2 found in the vicinity of redox switches and probes, based on their level of oxidation measured *in vivo*. This procedure improved previous estimations of H_2O_2 concentrations and gradients that were based on antioxidant enzyme activities determined *in vitro*.
Conclusion: The canonical model described here is a predictive and analytical tool that helps to depict the cellular hydrogen peroxide signaling landscape and holds the potential to be a framework for a future redox kinetomics analytical platform.
Acknowledgement: Fundação para a Ciência e Tecnologia (FCT) grant UID/QUI/00100/2019.

Symposium 13-1
“What, where and how much? Key challenges in protein oxidation”

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Protein oxidation is a frequent event as a result of the high abundance of proteins in biological samples and the multiple processes that generate oxidants. The reactions that occur are complex and poorly understood, but can generate major structural and functional changes on proteins. Considerable data indicate that pathophysiological processes and multiple human diseases are associated with the accumulation of damaged proteins.
Whilst there is therefore abundant evidence for the occurrence of protein modifications in human physiology and pathology whether this is causative in particular events remains more problematic to determine. This is due, at least in part to a lack of information as to the precise nature of the modifications that are present on proteins (in many cases only a partial picture is available, due to the use of assays that only provide limited or non-specific information), where these modifications occur within a protein sequence (and hence whether any particular modification is of functional significance), and the extent of modification – both overall and at particular locations.
Recent advances are providing critical clues as to the nature, sites and abundance of particular modifications induced by specific oxidants, but this data remains limited. There is a clear need to develop better tools and approaches to allow a quantitative assessment of the role of protein modification in both health and disease, and allow critical judgements to be made as to whether protein alterations are causative in disease, or merely collateral damage. These topics will be addressed

in this presentation with a particular emphasis on damage induced by oxidants generated at sites of chronic inflammation, and damage to disulfide bonds in extracellular proteins.

Symposium 13-2
Modifications of cysteine residues in the generation of structurally and functionally diverse protein species

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Cysteine residues in proteins can be the targets for enzymatic and non-enzymatic modifications that generate a great structural and functional diversity. Through the moieties attached to cysteine residues proteins can gain hydrophobicity involved in their subcellular localization or secretion, acquire or loose enzymatic activity or undergo conformational changes altering the assembly properties of polymeric structures, leading to functional cytoskeletal rearrangements or aberrant aggregates. Cysteine residues are critical sensors of the cellular redox status and their modification plays a key role in activating the cellular antioxidant defences. Moreover, there can be interplay between enzymatic and non-enzymatic modifications resulting in fine tuning of protein properties. We have been interested in identifying cysteine residues that are targets for adduction of electrophilic lipids under various conditions, and in exploring the functional consequences that these modifications bring about in the protein targets. Control studies employing cysteine deficient mutants have led us to the identification of certain cysteine residues that are key for the normal function of the corresponding proteins. Here we will focus on the single cysteine residue of certain cytoskeletal proteins of the intermediate filament family, which acts as a hinge affecting protein assembly and function in a manner depending on the structure of the modifying moiety. We will illustrate how this cysteine residue confers these proteins a role as sensors of redox and electrophilic stress, transducing changes in cellular redox status into cytoskeletal responses.
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Symposium 13-3

Lipid oxidation products induce specific protein modifications: biological effects and analysis by LC-MS/MS

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Lipid peroxidation leads to formation of a variety of reactive products, including short-chain aldehydes such as acrolein, malondialdehyde, pentanal, 4-hydroxyhexanal (HHE), and the esterified product palmitoyloxovaleroyl phosphatidylcholine (POVPC). These compounds have a variety of biological effects relating to inflammation. One mechanism involved is the formation of covalent adducts with proteins, a process called lipoxidation. The precise effects of these modifications are protein and adduct specific; protein function is often altered, for example inhibition of enzymes, aggregation and enhanced proteolysis, although in a few cases activity may be increased. Analysis of the location of the modification of individual proteins is challenging and requires advanced liquid chromatography mass spectrometry (LC-MS) techniques. We have focused mainly on the development of label-free mass spectrometry methods for the identification of aldehyde adducts, initially using model proteins such as lysozyme, human serum albumin and Apo B-100. Mapping the sites of modifications by short-chain esterified and non-esterified aldehydes, monitoring the dose-dependent response and comparing the susceptibility of residues to different aldehydes have improved our understanding of protein structure-function relationships during lipoxidation. These experiments also allowed the identification of diagnostic ions specific for aldehyde adducts, which can be used for multiple reaction monitoring (MRM) and precursor ion scanning mass spectrometry detection approaches in more complex samples such as human plasma and cell extracts. This lecture will provide an overview of the ongoing successes and challenges in the analysis of lipoxidation and its effects, illustrated by key biological examples.
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Symposium 13-4

Oxidative protein modifications of protein therapeutics: targeted proteomic analysis and consequences for stability, efficacy and immunogenicity

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Protein oxidation represents a major degradation pathway affecting the stability of protein therapeutics. Generally, oxidation reactions are carried out by reactive oxygen species (ROS), which can be generated in pharmaceutical formulations via multiple pathways. Moreover, oxidation products from all constituents of a formulation can react with proteins to generate secondary products. Based on the manifold of potential ROS and oxidation products, we expect to see on the order of 10^2-10^3 different protein degradation products under oxidizing conditions, depending on the size of the protein, though the relative yields of individual products may be low. Nevertheless, even low yields of specific oxidation products may be of concern if these products would trigger an immune response. In an attempt to characterize all reaction products arising from oxidative degradation, we subjected proteins (human growth hormone, IgG1, IgG1-Fc, IgG4-Fc) to targeted proteomic analysis. We first characterized a series of hitherto unknown covalent chemical cross-links induced by the exposure to light, i.e. the formation of dithiohemiacetal, thiooether, ether and vinyl ether cross-links. Structurally, these cross-links are different from His-His cross-links, also generated during the exposure to light, while parallels exist with regard to the mechanisms of formation. Second, we characterized novel fragmentation mechanisms triggered by the side chain cleavage of Trp and Tyr. These reactions convert Trp and Tyr into either Gly, or lead to protein backbone fragmentation. Even the conversion of Trp or Tyr to Gly has a significant impact on thermostability and receptor binding, for example of IgG4-Fc. Moreover, the side chain cleavage products of Trp and Tyr, 3-methyleneindolenine and quinone methide, are powerful electrophiles, which react efficiently with nucleophilic amino acid side chains to generate novel epitopes. During these studies we also discovered that tungstate, even at trace levels, can significantly accelerate protein fragmentation.

ABSTRACTS of SFRR-INTERNATIONAL 2021 VIRTUAL MEETING

Oral Presentations

OP1
Adduction reactions of alpha,beta-unsaturated carbonyls: using kinetics to determine biologically relevant reactions.

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University of Copenhagen, Copenhagen, Denmark

Humans are exposed to a wide range of alpha,beta-unsaturated carbonyls (ABuCs). Thus, acrolein is a major toxin in cigarette smoke and automobile exhausts, and a product, together with others (e.g. crotonaldehyde, 4-hydroxynonenal, 4-hydroxypentenal) from polyunsaturated fatty acid peroxidation. Other ABuCs have positive actions, with dimethylfumarate used to treat psoriasis and multiple sclerosis. Understanding the effects of these species is therefore important for toxicology and drug development. ABuCs undergo Michael addition reactions with biological nucleophiles including DNA bases and protein side-chains; the latter are a major target due to their abundance. Although reaction can occur with amine (e.g. Lys) and imidazole (His) groups, adduction to Cysteine (Cys) typically dominates. The kinetics and selectivity of these processes are incompletely understood. In this study we aimed to determine kinetic data for reaction with Cys residues, information on the selectivity of adduction, and the factors that control these. Rate constants and downstream effects on protein function (enzymatic assays) have been determined for multiple AbuCs. Rate constants for addition of *N*-Ac-Cys to acrolein, crotonaldehyde, dimethylfumarate, cyclohexanone and cyclopentenone vary by 250-fold indicating that AbuC structure is a key determining factor, with acrolein being the most reactive. Values for GSH vary by 350-fold. Cys groups in different proteins react at different rates. These rates are also greater (up to 10-fold) than for *N*-Ac-Cys and GSH, rationalizing the detection of protein adducts within cells, despite the high intracellular GSH concentration. The rate constants show a linear inverse correlation with thiol pKa, indicating that low pKa Cys residues on proteins (i.e. those present predominantly as RS^-) are major sites of reaction. Assays of enzyme activity, indicate that adduction at Cys residues is strongly associated with a loss of enzyme activity, showing that these reactions have functional consequences.
Keywords: Alpha,beta-unsaturated aldehyde, Acrolein, Dimethylfumarate, protein modification, Michael reaction

OP2
A functional analysis to dissect the role of ROS signaling pathways using chemogenetic tools and genetically encoded biosensors for in vitro studies

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Hydrogen peroxide (H_2O_2) is a versatile signaling molecule that belongs to the most studied reactive oxygen species (ROS) in biology. At higher concentrations, it causes oxidative stress while lower concentrations of H_2O_2 modulate intracellular signaling pathways. However, for many decades H_2O_2 signaling pathways have been examined by the administration of physiologically irrelevant levels of H_2O_2 to living cells and tissues. Owing to the lack of suitable tools, the role of endogenous H_2O_2 remained a mystery and led to contradictory results. Here we exploit a yeast-derived D-amino acid oxidase (DAAO) as a chemogenetic tool to generate on-demand intracellular H_2O_2 production with high spatial and temporal resolution. This yeast enzyme generates H_2O_2 only in the presence of D-amino acids. The provision or withdrawal of D-amino acids can thereby modulate oxidant-regulated pathways. The novel genetically encoded HyPer7 biosensor permits the simultaneous detection of the DAAO derived H_2O_2. Here we have thoroughly characterized the functionality of the DAAO enzyme in different cell lines and subcellular locales. For this purpose, we tested various substrates (D-amino acids) in terms of their catalytic activity. This approach unveiled that D-methionine as a substrate yields significantly faster H_2O_2 generation compared to commonly used substrates such as D-alanine. Exploiting the well-established hsGFP biosensor for hydrogen sulfide we demonstrated the DAAO enzyme yields H_2S in the presence of sulfuric amino acid D-Cysteine, however, not in the case of D-Methionine. In this study, we provide fine-tuned strategies and protocols for the precise control of DAAO in cultured cells. We anticipate that our results and assays will allow scientists to dissect the role of H_2O_2 under controlled and more physiologically relevant conditions.
Keywords: Hydrogen peroxide, D-amino acid oxidase, chemogenetics, HyPer

OP3
Identification of SELENBP1 as a hydrogen sulfide source in intestinal epithelial cells through a novel methanethiol oxidase assay

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Methanethiol, a gas with the characteristic smell of rotten cabbage, is a product of microbial methionine degradation. In humans, most of the methanethiol originates from the gut microbiome, and highest levels are measured in the intestine and in blood. Selenium-binding protein 1 (SELENBP1) has recently been identified as a methanethiol oxidase (MTO), catalyzing the conversion of methanethiol to hydrogen sulfide (H_2S), hydrogen peroxide (H_2O_2) and formaldehyde. Single nucleotide polymorphisms in the *SELENBP1* gene were linked to extraoral halitosis, caused by loss of MTO function.
Here, human Caco-2 intestinal epithelial cells were subjected to differentiation from crypt- to villous-like enterocytes, followed by analysis of SELENBP1 levels and MTO activity. To that end, we established a novel coupled assay to assess MTO activity in a way mimicking the proximity of bacteria and intestinal epithelial cells *in vivo*. The assay is based on *in situ*-generation of methanethiol from methionine as catalyzed by a bacterial recombinant L-methionine gamma-lyase (MGL), followed by detection of two of the three methanethiol oxidation products, H_2S and H_2O_2. Whereas H_2S is detected through precipitation as lead sulfide, which can be assessed colorimetrically, H_2O_2 is detected enzymatically using horseradish peroxidase and a fluorimetric cosubstrate.
First, we established the MTO assay, using human SELENBP1 as well as inactive mutants thereof (His329Tyr; Gly225Trp) that were produced in *E. coli* as recombinant proteins. Then, we demonstrated that MTO activity was strongly enhanced in Caco-2 cells upon differentiation, in parallel with an increase in SELENBP1 protein levels. This suggests that differentiated intestinal epithelial cells, rather than their precursors, are capable of eliminating methanethiol of microbial origin.
This study was supported by the German Research Foundation (DFG), RTG 2155/2 “ProMoAge”.

OP4
Identification of an endogenous long non-coding RNA that inhibits selenoprotein P translation- New regulatory mechanism of SeP translation

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Selenoprotein P (SeP) is a major plasma selenoprotein that contains 10 selenocysteine (Sec) residues, which is encoded by the UGA stop codon. The mRNA for SeP has the unique property of containing two Sec insertion sequence (SECIS) elements, which is located in the 3' untranslated region (3'UTR). SeP functions as a selenium (Se)-supply protein to maintain Se in the several tissues including the brain and testis. Further, SeP is identified as a hepatokine, promoting insulin resistance in type 2 diabetes (Cell Metabolism 2010). Thus, the suppression of Se- supply activity of SeP might improve glucose metabolism (Nature Commun 2017). Here, we coincidentally identified a novel gene, *CCDC152*, by sequence analysis. This gene was located in the antisense region of the *SeP* gene, including the 3'UTR region in the genome. We demonstrated that this novel gene functioned as a long non-coding RNA (lncRNA) that decreased SeP levels via translational rather than transcriptional, regulation. We found that the *CCDC152* RNA interacted specifically and directly with the SeP mRNA and inhibited its binding to the SECIS-binding protein 2 (SBP2), resulting in a decrease of ribosome binding. We termed this novel gene product lncRNA inhibitor of SeP translation (*L-IST*). Finally, we found that several compounds upregulated *L-IST* in vitro and in vivo, to suppress SeP levels. Here, we provide a new regulatory mechanism of SeP translation by an endogenous long antisense ncRNA. Translational control of SeP by *L-IST* may be a new therapeutic strategy for SeP-related diseases such as diabetes and pulmonary hypertension.

OP5
Na^+ controls hypoxic signalling by the mitochondrial respiratory chain

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All metazoans depend on the consumption of O_2 by the mitochondrial oxidative phosphorylation system (OXPHOS) to produce energy. In addition, the OXPHOS uses O_2 to produce reactive oxygen species that can drive cell adaptations[1-4], a phenomenon that occurs in hypoxia[4-8] and whose precise mechanism remains unknown. Ca^{2+} is the best-known ion that acts as a second messenger[9], yet the role ascribed to Na^+ is to serve as a mere mediator of membrane potential[10]. Here we

show that Na^+ acts as a second messenger that regulates OXPHOS function and the production of reactive oxygen species by modulating the fluidity of the inner mitochondrial membrane. A conformational shift in mitochondrial complex I during acute hypoxia[11] drives acidification of the matrix and the release of free Ca^{2+} from calcium phosphate (CaP) precipitates. The concomitant activation of the mitochondrial Na^+/Ca^{2+} exchanger promotes the import of Na^+ into the matrix. Na^+ interacts with phospholipids, reducing inner mitochondrial membrane fluidity and the mobility of free ubiquinone between complex II and complex III, but not inside supercomplexes. As a consequence, superoxide is produced at complex III. The inhibition of Na^+ import through the Na^+/Ca^{2+} exchanger is sufficient to block this pathway, preventing adaptation to hypoxia. These results reveal that Na^+ controls OXPHOS function and redox signalling through an unexpected interaction with phospholipids, with profound consequences for cellular metabolism.
Hernansanz-Agustín, Pablo et al. "Na^+ controls hypoxic signalling by the mitochondrial respiratory chain." *Nature* vol. 586,7828 (2020): 287-291. doi:10.1038/s41586-020-2551-y

OP6
Mitochondrial cristae shape regulates ROS production under restrictive glycolysis

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Mitochondrial cristae are membrane platforms where respiratory (super)complexes embed to account for oxidative phosphorylation and energy production. Despite growing evidences supporting a key role for cristae shape in determining supercomplex stability and hence respiration, it is still poorly understood whether and how the inner mitochondrial membrane links bioenergetic demands to reactive oxygen species (ROS) generation. Here we report that forced ATP fueling by mitochondria under blunted glycolysis, impacts F_1F_O-ATP synthase oligomerization and cristae morphology. Capitalizing on genetic and apoptotic models of cristae remodeling, we demonstrate that cristae loss and altered mitochondrial ultrastructure predispose to mitochondrial ROS generation and cell death. In this scenario, the master regulator of cristae shape Opa1 provides a scaffolding platform that prevents the collapse of cristae and mitochondrial bioenergetics, hence preventing ROS accumulation independently on changes in the cellular or mitochondrial antioxidant capacity. Both ROS generation and cell death are aggravated in conditions where the cristae scaffolding role of F_1F_O-ATP synthase dimers at tips or Opa1 at cristae junctions is disrupted, demonstrating that variations in mitochondrial ultrastructure are sufficient to accommodate ROS production to changes in the activity of the electron transport chain. Altogether, our results unravel a so far elusive mechanism linking bioenergetic demands and mitochondrial ultrastructure, at setting ROS levels under forced or compromised mitochondrial function.

OP7
Aberrant PFKFB3-induced glycolysis in neurons *in vivo* causes mouse behavioral impairment by mitochondrial redox stress

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Our group is interested in understanding the mechanisms that contribute to the metabolic and redox coupling between astrocytes and neurons, as well as their implication in cognitive and motor functions. Neurons show a high dependence on mitochondrial oxidative phosphorylation for survival, whereas astrocytes resist to almost complete inhibition of mitochondrial respiration. A key factor in this process is PFKFB3, an enzyme that promotes glycolysis by activating its regulatory enzyme PFK1. We previously demonstrated that PFKFB3 is a substrate of the E3 ubiquitin ligase APC/C-Cdh1. By degrading PFKFB3, APC/C-Cdh1 activity shifts the equilibrium of glucose consumption from glycolysis towards pentose-phosphate pathway (PPP), thus regulating the redox status and survival of neurons. Conversely, APC/C-Cdh1 activity in astrocytes is very low, which accounts for the elevated PFKFB3 protein levels and glycolytic phenotype of these cells. However, the impact of PFKFB3 modulation in specific brain cells on cognitive and motor functions have not

yet been explored. To address this, we generated a Cre recombinase-inducible ROSA26-floxed knock-in mouse harboring the full-length PFKFB3 cDNA (PFKFB3$^{LoxP/+}$) to specifically assess the impact of PFKFB3 expression in neurons *in vivo*. PFKFB3$^{LoxP/+}$ mice were crossed with mice expressing Cre recombinase under the neuronal CAMKIIa promoter (CAMKIIa-Cre). We found that the resulting PFKFB3$^{LoxP/+;\ CAMKIIa\text{-}Cre}$ mice showed increased PFKFB3 protein levels in neurons *in vivo* and symptoms of cognitive decline and motor discoordination as from the 3 months of age. Interestingly, this PFKFB3-induced phenotype was rescued by *in vivo* co-expressing mitoCatalase (mCAT), i.e. the antioxidant enzyme catalase confined to neuronal mitochondria. These results indicate that aberrant enhancement of glycolysis in neurons causes behavioral impairment in mouse through a mitochondrial redox stress-mediated mechanism.

OP8

Does the mitochondrial import and assembly (MIA) pathway contribute to the hydrogen peroxide production in mitochondria?

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Introduction: Erv1 (called ALR in human) is an essential mitochondrial enzyme, which works together with Mia40 catalysing the import and oxidative folding of newly imported proteins in the mitochondrial intermembrane space. Erv1/ALR is a FAD-dependent disulphide bond generating enzyme with both oxidase and cytochrome *c* reductase activities. As an oxidase, Erv1/ALR passes electrons from a thiol substrate to molecular oxygen (O_2) with production of hydrogen peroxide (H_2O_2), and as a cytochrome *c* reductase, it passes electrons to cytochrome c of the electron transport chain. The short form of human ALR (sfALR) prefers cytochrome *c* as a substrate *in vitro*. However, the relative substrate preference for oxygen and/or cytochrome c of these enzymes in mitochondria is unknown. In this study, we investigated the oxidase and cytochrome *c* reductase activities of yeast Erv1, and how liposomes affect its function.
Material & methods: Using yeast Erv1 as a model, the enzyme kinetics of its oxidase and cytochrome c reductase were analysed, using absorption spectroscopic methods and oxygen consumption assays, in the absence and presence of liposomes that mimic mitochondrial membranes.
Results**:** In the absence of liposomes, cytochrome *c* was an approximately 10-fold more efficient substrate than O_2 for yeast Erv1, showing that Erv1 is a better cytochrome *c* reductase than oxidase. However, in the presence of liposomes, whilst liposomes had a mild effect on Erv1 oxidase activity, they inhibited the catalytic efficiency of its cytochrome *c* reductase activity strongly and in a cardiolipin dependent manner.
Conclusion: (1) Erv1 is a moderately active enzyme with the ability to use both O_2 and cytochrome *c* as electron acceptors *in vitro*. (2) Erv1 may be a better oxidase than cytochrome *c* reductase in yeast mitochondria, suggesting that the MIA pathway contributes to mitochondrial hydrogen peroxide (H_2O_2) production in yeast.

OP9

An inhibitor of the NRF2-βTrCP interaction suppresses lipopolysaccharide-mediated inflammation *in vitro* and *in vivo*

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Most diseases are characterized by exacerbated inflammation that is controlled by corticosteroids and non-steroidal anti-inflammatory drugs. However, these drugs exhibit many undesired effects that preclude their long-term administration. A new strategy to control inflammation is the activation of transcription factor NRF2, nowadays considered as a master regulator of cellular homeostasis. From a clinical perspective, NRF2 activation produces a beneficial therapeutic effect in many diseases that present with oxidative and inflammatory stress. Intensive research has been focused on the identification of small molecule inhibitors of the E3 ubiquitin ligase KEAP1, which is the canonical mechanism for the ubiquitin-proteasome degradation of NRF2. However, a completely unexplored alternative is the pharmacological modulation of the E3 ubiquitin ligase β-TrCP. Here we report the development of a Protein-Protein Interaction (PPI) inhibitor of NRF2-β-TrCP that offers an alternative to KEAP1 for NRF2 activation. This small molecule increases NRF2 levels and induces the expression of NRF2-regulated genes such as *Hmox1*, in control and in KEAP1-defficient fibroblasts, but not in β-TrCP-knock-down cells. Moreover, the compound attenuates the production of pro-inflammatory markers in cultured

macrophages and in mice submitted to the endotoxin lipopolysaccharide. These findings suggest that this compound could be used as an alternative to conventional anti-inflammatory therapies.
Keywords: NRF2, b-TrCP, KEAP1, Protein-Protein Interaction (PPI) Inhibitor, Inflammation, LPS.

OP10
Modification of histones by hypochlorous acid and its influence on vascular cell function

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The release of neutrophil extracellular traps (NETs) is a key innate immune defense to combat infection. NETs consist of a mesh of DNA and histones, which are decorated with neutrophil granule proteins, including myeloperoxidase (MPO). Although NETs have important anti-bactericidal properties, they are also implicated in thrombosis and the development of atherosclerosis and other chronic inflammatory pathologies. However, the mechanisms involved in the pathological effects of NETs are not well understood. Enzymatically-active MPO is present on the DNA NET backbone, and produces the potent oxidant hypochlorous acid (HOCl). However, whether this results in the oxidative modification of NET components has not been assessed. In this study, we characterized the reactivity of HOCl with histone proteins, which are abundant in NETs, and assessed whether these modifications influenced the reactivity of histones with vascular cell models. Experiments were performed with a preparation of histones containing histone H1, H2A, H2B, H3 and H4. Treatment of the histones with HOCl resulted in the modification of Lys residues and the formation of unstable chloramines, which decomposed over 24 h. Evidence was also obtained for a dose- and time-dependent decrease in the concentration of Met, Arg and Tyr residues, which was accompanied by the formation of stable oxidation products, including Met sulfoxide, 3-chloro-Tyr and protein carbonyls. Exposure of model human vascular cells, including human coronary artery endothelial cells and human coronary artery smooth muscle cells, to non-modified histones resulted in a dose-dependent loss of viability, consistent with the known toxicity of histones when present in the extracellular environment. However, this loss in viability was attenuated on pre-treatment of the histones with HOCl, which was dependent on the extent of oxidative modification. This may be relevant in inflammatory pathologies such as atherosclerosis, where NETs are associated with lesion development and vascular dysfunction.

OP11
Ubiquitination as a key regulatory mechanism for O_3-induced cutaneous redox inflammasome activation

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Due to its location, skin tissue is one of the first organ exposed to the noxious effects of environmental pollutants, becoming one of the preferred target tissue. Among pollutants, Ozone (O_3) is one of the most toxic agents, able to initiate oxidative reactions within the skin tissue, triggering the activation of redox and inflammatory pathways (Oxinflammation). Inflammasomes are multiprotein complexes whom activation has been associated with the onset of inflammatory pathologies and also linked to air pollutants and UV exposure. NLRP1, one of the main inflammasomes found in the skin, has been recently shown to be susceptible to O_3 exposure and also related to several skin conditions. However, the mechanisms behind its aberrant activation are still not completely clear. In the present work, we demonstrated that O_3 is able to modulate cutaneous inflammasome activation (scaffold formation and release of the final mediator IL-1β) via a redox mechanism which, involves the O_3 oxidative mediators: hydrogen peroxide (H_2O_2) and 4-hydroxy-nonenal (4HNE). Inhibition of Interleukin-1 converting enzyme (ICE) Caspase 1 via Z-YVAD-fmk was able to prevent IL-1β release from human keratinocytes exposed to O_3, confirming the role of the pollutant in inflammasome activation. Moreover, we found that inflammasome components NLRP1 and ASC are targets for 4HNE adduction which is known to modify protein functionality and also lead to their degradation via different mechanisms, including the ubiquitin-proteasome pathway. Of note, exposure of human keratinocytes to O_3 induced high levels of ubiquitinated proteins, including NLRP1, while treatment with catalase was able to prevent not only NLRP1 ubiquitination but also NLRP1 scaffold formation and activation. In conclusion, our findings suggest that O_3 may be able to trigger NLRP1 inflammasome activation in a redox dependent manner via ubiquitination and possible degradation of its autoinhibitory N-terminal domain.

OP12
LSD1 contributes to defining pro-inflammatory phenotype of human macrophages by repressing catalase

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The specialization of monocytes into macrophages M1 and M2 occurs in two pathways: classical, (pro-inflammatory) and alternative (anti-inflammatory) depending on the environmental factors such as cytokines, agents produced by pathogens or fatty acids, and the stage of inflammation. The classic type of macrophages has significantly increased levels of hydrogen peroxide that might be explained by the activation of NADPH oxidases and mitochondrial superoxide dismutase 1, as well as by the suppression of catalase. Our results provide the evidence that the stimulation of TLR2 and TLR4 by their agonists (analogs of bacterial molecular patterns) Pam3CSK4 and LPS, respectively, leads to the CAT repression in LSD1-dependent fashion. LSD1 is recruited to the *CAT* promoter in response to TLR activation and decreases transcription-promoting methylation of H3K4. Silencing of catalase with siRNA increases transcription of cytokines such as *IL1β*, *MIP2A*, *COX2* without cell stimulation with LPS, whereas the maintenance of catalase level with LSD1 inhibitors substantially reduces expression of the above listed pro-inflammatory factors. These findings lead to the conclusion that LSD1-mediated repression of *CAT* during macrophage M1 polarization facilitates gaining of pro-inflammatory phenotype.

OP13 Adaptation of bEnd.3 brain microvascular endothelial cells to physiological normoxia reduces superoxide production associated with reperfusion injury

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Treatments available for ischemic stroke remain limited due to failures in clinical translation and experiments *in vitro* need to recapitulate physiological O_2 levels encountered *in vivo*. As cells *in vivo* experience O_2 levels ranging from ~13 kPa to ~1 kPa, cells cultured under room air (18 kPa O_2) are exposed to hyperoxic stress. Using an O_2-sensitive probe (MitoXpress-INTRA, Agilent), an intracellular O_2 level of 3.6 kPa was measured in murine brain endothelial cells (bEnd.3) cultured long-term under 5 kPa O_2 in an O_2-regulated workstation (Baker-Ruskinn), recapitulating intracellular O_2 levels reported in the brain. Long-term culture under 5 kPa O_2 results in a phenotype different to cultures under 18 kPa O_2, as evidenced by downregulation of specific Nrf2 target antioxidant genes. bEnd.3 cells cultured either under 18 kPa O_2 (hyperoxia) or 5 kPa O_2 (physiological normoxia) were subjected to hypoxia-reoxygenation to model transient ischemic stroke, and superoxide production was measured using L-012 in real time in an O_2-regulated plate reader (BMG Labtech). Adaptation of bEnd.3 cells to 5 kPa O_2 attenuated superoxide production induced by reoxygenation, suggesting that exaggerated radical generation in cells exposed to hyperoxia is due to elevated oxidative stress. Our findings highlight the importance of conducting vascular cell culture under physiological normoxia to recapitulate the redox phenotype of brain microvascular endothelial cells *in vivo*.
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OP14 The nitrate-nitrite-nitric oxide pathway improves neurovascular coupling under cerebral hypoperfusion conditions

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The functional and structural integrity of the brain rely on a prompt delivery of metabolic substrates via local changes in cerebral blood flow, matching the increase in neuronal activity – neurovascular coupling (NVC). A dysfunctional NVC is an early event in the toxic cascade leading to neurodegeneration. Considering that neuronal-derived nitric oxide ($^{\bullet}NO$) is a direct mediator of NVC in the hippocampus, it is hypothesized that dietary modulation of circulating nitrite levels may convey a pathway to sustain NVC under hypoxic conditions, via redox cycle of nitrite with ascorbate, yielding $^{\bullet}NO$ in the brain extracellular space.

To test this hypothesis, we studied the impact of acute administration of nitrite and ascorbate in the NVC in a rat model of cerebral hypoperfusion (bilateral occlusion of the common carotid arteries - 2VO) *in vivo*. Specifically, the hemodynamic responses to glutamatergic activation were characterized by Laser Doppler flowmetry upon sustained hypoperfusion and the modulatory effect of nitrite/ascorbate addressed after a bolus intracerebroventricular injection.
Data supports a significant impairment in the NVC in the acute phase of cerebral hypoperfusion (up to 3h). In the hippocampus, 2VO promotes a sustained decrease of cerebral perfusion (ca.35%) that occurs along with a local decreased of extracellular pH and O_2. Concomitantly, the spatially restricted hemodynamic response to local glutamatergic activation after 2VO is dramatically different from control responses: the amplitude of the CBF response to stimulation is ca.40% smaller and its duration is ca.60% longer. Interestingly, the acute administration of nitrite and ascorbate promoted an increase in the hippocampal blood perfusion and modulated the hemodynamic response to neuronal activation (increased the temporal profile).
Overall, our results support that, upon glutamatergic stimulus *in vivo*, ascorbate-driven reduction of nitrite to $^{\bullet}$NO in the brain improve NVC under cerebral hypoperfusion conditions.
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OP15
Neuron specific lack of SOD1 leads to accelerated age-related loss of NMJ structure

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Sarcopenia is accompanied by structural abnormalities in neuromuscular junctions (NMJs). Mice with whole body deletion of Sod1 mimic this process, but it is not clear what role a specific loss of Sod1 in neurons plays in the degeneration. In order to discover whether a specific loss of Sod1 in motor neurons accelerated loss of neuromuscular function this work examined measures of oxidation and the accumulation of NMJ structural alterations in an inducible motor neuron Sod1KO (i-mnSod1KO) mouse model. Mice studied were adult (8-10months), mid-age (16-18months) and old (24-27months) wild type (WT), mid-age and old i-mnSod1KO, whole body Sod1KO and SynTgSod1KO (whole body Sod1KO with nerve Sod1 expression rescued).
The activities of ROS in sciatic nerve were quantified using electron paramagnetic resonance (EPR) following infusion with a CPH spin probe. A significant increase in the EPR signal from the sciatic nerve of Sod1KO mice was seen compared with adult WT mice and i-mnSod1Ko mice at any age. Western blot analysis of sciatic nerves showed no significant difference in markers of oxidative damage (3-NT and carbonyl contents) between groups.
NMJ structure was assessed in terms of overlap, fragmentation and complexity and showed that at mid-age there was no significant impact on NMJ structure compared with adult WT. There was a decline in NMJ structure with advancing age in both WT and i-mnSod1KO mice with a higher incidence of denervated NMJs and a lower level of complexity in NMJs of old i-mnSod1KO mice compared with age-matched WT.
In conclusion, an alteration in redox homeostasis in the motor neuron did not result in detectable oxidative damage. Data suggest that this may alter re-innervation capability of the nerve beyond that observed with ageing, although a significant phenotype is not seen until other age-related changes accumulate.
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OP16
Impact of SAPAP3 on mitochondrial function in Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion at *HTT* gene, also characterized by motor and cognitive impairment and early psychiatric disturbances (e.g. obsessive-compulsive disorder -

OCD). Mutant huntingtin (mHTT) affects striatal GABAergic neurons and glutamatergic cortico-striatal synapses and causes, among other hallmarks, mitochondrial dysfunction. Previous studies demonstrated that the postsynaptic scaffold protein SAPAP3, mainly located in striatum, is an important player in OCD. Preliminary data indicate that this protein has several mitochondrial interactors. Therefore, striatal dysfunction linked to early mitochondrial deregulation may involve changes in SAPAP3, and potentially explain HD-related psychiatric disturbances. We analyzed SAPAP3 protein and mitochondrial levels in pre-symptomatic (3 m.o.) and symptomatic (6, 10-12 m.o.) YAC128 transgenic (expressing full-length mutant HTT) *versus* WT mice, primary striatal and cortical cultures from YAC128 *versus* WT mice and immortalized mouse mutant striatal cells derived from HD knock-in mice (ST*Hdh*$^{Q111/Q111}$) *versus* WT (ST*Hdh*$^{Q7/Q7}$) cells. Moreover, we studied SAPAP3 involvement on mitochondrial function and dynamics, by both silencing and overexpressing SAPAP3. Our results showed reduced SAPAP3 total and mitochondrial levels in symptomatic YAC128 mice, mature primary neurons from YAC128 mice and ST*Hdh*$^{Q111/Q111}$ cells, compared to respective controls. Interestingly, in YAC128 primary striatal and cortical neurons, SAPAP3 diminished levels were pronounced at distal neurites, pointing towards a postsynaptic deregulation in HD. Accordingly, colocalization between SAPAP3 and an important scaffold protein, PSD-95, demonstrated decreased puncta number and area, as well as altered SAPAP3 levels. Of relevance, SAPAP3 was shown to be involved in normal mitochondrial function. Silencing SAPAP3 impaired mitochondrial morphology (e.g. increased roundness), neurite mitochondrial movement and function, and generated higher levels of reactive oxygen species. SAPAP3 overexpression ameliorated all these mitochondrial phenotypes in HD cells. Our data indicate that SAPAP3 levels control mitochondrial function and that targeting this protein might have a neuroprotective role in HD.

OP17
Olive oil and exercise training ameliorate muscle mitochondrial homeostasis in rats fed a high-fat diet

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Purpose: To investigate the effect of a 12-week EVOO alone and in conjunction with endurance training on mitochondrial (mt) homeostasis in rats fed a high-fat diet.
Methods: Female Sprague-Dawley rats, aged 4-6 weeks, were randomly divided into 4 groups: 1) Standard chow diet (C, N=6); 2) fed a high-fat/high-cholesterol atherogenic diet (Ath, N=12); 3) fed the Ath diet with EVOO (EVOO, N=12); and 4) fed the Ath+EVOO diet with exercise training (25 m/min, 10% grade for 60 min/day, for 12 wks; EVOO+T, N=12).
Results: CS activity and COX4 level were lower in Ath vs. C (P<.05), but restored by EVOO+T (P<.01). Ath increased levels of atrogin-1 and MuRF1, protein ubiquitination (Ub, P<.01), whereas the effect was abolished with EVOO and EVOO+T. Ath decreased OPA1 (P<.05), while DRP1 and Fis1 levels were increased with Ath (P<.05). EVOO increased Mfn2 and OPA1 levels (P<.01), whereas Fis1 level was decreased (P<.05). EVOO+T further increased Mfn2 and OPA1 vs. C and Ath (P<.01). DRP1 level was decreased in EVOO+T vs. Ath and EVOO (P<.01), but training increased Fis1 level vs. C and EVOO (P<.05). PINK1 level was decreased in Ath vs. C (P<.05), EVOO and EVOO+T elevated PINK1 level (P<.01). SOD2 level was not affected by Ath, but was upregulated by EVOO and EVOO+T (P<.01). Catalase level increased in Ath vs. C (P<.01) and was further elevated with EVOO and EVOO+T (P<.01). Ath increased GPx1 level vs. C (P<.05), which was further elevated by EVOO (P<.01). 4HNE content was increased in Ath and EVOO (P<.01) vs. C, but was reduced by EVOO+T (P<.05).
Conclusion: Ath impaired muscle mitochondrial homeostasis due to altered dynamics and mitophagy. EVOO supplementation partially reduced Ath adverse effects, while exercise training provided additional protection against the Ath effects.

OP18
The role of extracellular vesicles in the regulation of redox homeostasis during exercise: a focus on Nrf2 and antioxidant enzymes

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The transcription factor Nrf2 (NF-E2-related factor 2) plays an important role in maintaining Redox homeostasis (RH) by regulating multiple downstream antioxidants. The role of physical exercise to trigger Nrf2, in response to the increase in ROS, is already known. Extracellular Vesicles (EVs) are a heterogeneous collection of membrane-bound carriers with

complex cargoes, including proteins, lipids, and nucleic acids. Given the role of RH in exercise-induced signaling and adaptation, we focused on the study of the exercise-related intercellular communication of redox components mediated by EVs, including upstream and downstream factors. Plasma EVs have been isolated from trained and untrained healthy males (n=14, 20-35 years) before and after (3 and 24 hours) an acute bout of endurance exercise (70% HRmax for 30'), or a short-term endurance training (70% HRmax for 30'/day for 5 consecutive days), and analyzed for their content in Nrf2, Catalase, Glutathione Peroxidase 1, Thioredoxin reductase 1, Thioredoxin 2, SOD1, SOD2, p-38 MAPK, carbonylated protein and lipid peroxidation (4-HNE). Our results showed that plasma EVs contain discrete amount of Nrf2 and antioxidant enzymes, differently modulated by the fitness level and the exercise regime. While no specific modulation was detected for the Nrf2 content in EVs, our data highlighted that SOD2 (p=0.05) and CATALASE (p=0.005) content in EVs' cargo is decreased in trained with respect to the untrained subjects, with no effects exerted by the acute bout of exercise. When untrained subjects were submitted to 5-days of endurance training, CATALASE (p=0.05) and SOD2 (p=0.05) content were decreased, reaching levels similar to those found in trained subjects. This study shows the presence of Nrf2 and antioxidant enzymes in plasma EVs, indicating a cross-tissue molecular system to maintain and restore redox homeostasis, and possibly to counteract at systemic level the oxidative stress derived by poor fitness level or during physical exercise.

OP19
Small molecule-mediated promotion of healthspan through activation of redox-sensitive transcription factors in *C. elegans* and mice

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Within a rapidly aging society, age-associated diseases are increasingly occurring. Activation of redox-sensitive transcriptional regulators, including Nuclear factor erythroid 2-related factor 2 (NRF2) / nuclear factor erythroid-derived 2-like 2 (NFE2L2), are known to be instrumental in delaying such diseases. In this study, Nrf2 activating small molecules were identified in a cell-based assay by screening a library of more than 2,500 single substances, among them phytochemicals and internationally approved drugs. Subsequently, the nematode *C. elegans* was supplemented with the top Nrf2 activators and potential effects on lifespan were investigated. As a result, a structurally yet un-described compound derived from the root of *Daucus carota*, hereafter named *Carrot III*, was revealed as most promising for further analyses. On a molecular level, *Carrot III* affects cellular respiration by interacting with a component of the mitochondrial electron transport chain. On a phenotypical level, this results in an enhanced performance of *C. elegans* in various health assays (motility and stress assay) as well as disease models (Alzheimer's & Huntington's disease). Supplementation of the compound to wild-type C57bl6 mice on high fat diet revealed possible health promoting effects including decreased fasted blood glucose levels and increased exercise endurance. Based on the data obtained so far and depending on ongoing investigations, *Carrot III* might become a promising substance for further human studies to delay or prevent aging-associated diseases.

OP20
Impact of sulforaphane on genome integrity

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Background: Sulforaphane (SFN), an isothiocyanate contained in plants in the Brassicaceae family, has a variety of physiological activities including its antioxidant effect, apoptosis induction effects and radiation sensitization effects. Previous studies have shown that SFN has an activity to suppress the DNA repair machinery. However, such cytotoxic mechanisms of SFN have not been fully elucidated. In this study, we analyze the cytotoxic mechanisms of SFN to clarify the impact of SFN on the maintenance of genome integrity.
Methods: *Colony formation assay*: To examine the cytotoxic effect of SFN, HeLa cells were pretreated with various concentrations of SFN for 1 hour and cultured in growth medium containing hydrogen peroxide. To examine the involvement of SFN in DNA damage induction, HeLa cells were pretreated with NU7026, an inhibitor of DNA-dependent protein

kinase, and cultured with various concentrations of SFN. After two weeks, colonies were stained with crystal violet, and counted. *GSH/GSSG assay:* GSH/GSSG was quantified using the GSH/GSSG-Glo Assay Kit.
Results: Cell survival rate of hydrogen peroxide-treated HeLa cells was increased when cells were pretreated with a low concentration of SFN. However, the antioxidant effect of SFN was decreased in a concentration-dependent manner. Interestingly, NU7026-treated cells were more resistant to SFN than untreated cells, suggesting that SFN induces DNA single-strand breaks. As SFN binds to glutathione, we speculated that SFN modulates glutathione balance. To test this possibility, we quantified cellular GSH/GSSG and found that GSH was decreased after treatment with SFN, suggesting that decreased GSH led to accumulation of reactive oxygen species and resulted in accumulation of DNA single-strand breaks.
Conclusion**:** We concluded that SFN contributes to the maintenance of genome integrity through its antioxidant effect in low concentrations while genome instability can occur in cells treated with high concentrations of SFN.
Keywords: sulforaphane, antioxidant, DNA damage

OP21
Mice lacking Grx2 in mitochondria exhibit a metabolic phenotype

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Glutaredoxin 2 (Grx2) is a small redox-active enzyme belonging to the thioredoxin-fold family of proteins. The (de)glutathionylation of target proteins and the coordination of an iron-sulfur cluster in a holo-dimeric structure are the principal activities of this enzyme. In mice, glutaredoxin 2 is present in two major splicing variants: Grx2a that resides in the mitochondrial compartment, and Grx2c, which has a cytosolic localization. Herein we characterize a C57BL6J mouse model selectively depleted of Grx2 in mitochondria (mitochondrial Grx2 depleted or mGD). When compared to wild-type mice, mGD animals display an increased body weight and augmented plasma lipid levels without differences in the food intake. In addition, mGD liver is enlarged, characterized by greater lipid deposition and by an altered expression of enzymes involved in lipid metabolism. Furthermore, it presents a lower capacity of glycogen storage in comparison to the wild type liver. Regarding the effects of Grx2 depletion on mitochondrial activity, despite an unexpected lack of significant differences in the inner redox balance (total thiols, glutathione levels, activity of thiol redox enzymes), mGD liver mitochondria present an altered morphology, produce more ROS, show decreased oxygen consumption rates and display lower mitochondrial membrane potential. Thus, in mice, the lack of Grx2 in the mitochondrial compartment impairs the normal mitochondrial activity with the development of a peculiar metabolic phenotype in which alterations of the lipid handling capacity can be envisaged.

OP22
Reductive stress, complex I hyperactivities, and diabetes

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NADH overloading pressure in certain tissues in diabetes gives rise oxidative stress. As mitochondrial complex I is the major site accepting NADH electrons, how complex I responds to this reducing power and the consequences of this response have not been investigated. Therefore, we aimed to study the effects of NADH overloading on complex I and mitochondria in diabetes.
Materials and Methods: Type diabetic animal models such as db/db mouse and Zucker diabetic rat were purchased from Charles River. Type I diabetes was induced in rats by streptozotocin injection (I.P., 60 mg/kg). Complex I activity was measured by blue native gel electrophoresis. NADH content, ATP levels, cell death were measured using commercially available kits, respectively. Protein carbonyls, lipid peroxidation, and H_2O_2 were also measured.
Results: Pancreatic mitochondrial complex I was found to be highly upregulated by diabetic hyperglycemia in both type I and type 2 diabetes. Moreover, ATP levels were decreased while NADH content was increased. We also found that NADH pressure on complex I leads to oxidative stress that increased mitochondrial dysfunction and cell death.
Conclusion: Our study suggests that inhibition of complex I upregulation may be a novel approach to fighting diabetes.
Keywords: Reductive stress, complex I, mitochondria, diabetes

OP23

Impaired lung redox metabolism and cardiac mitochondrial function aggravates myocardial infarction in a mice model of chronic exposure to urban air pollution

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The World Health Organization estimates that 91% of the world's population breathe low quality air. As a consequence, 7 million premature deaths occur every year due to air pollution exposure. From those, myocardial infarction (MI) accounts for 2.4 million deaths yearly, which represents 25% of the total global burden for this disease. Here, we aimed to understand some of the mechanisms by which urban air pollution exposure aggravates MI, focusing on the effects of airborne fine particulate matter (PM2.5) on lung redox metabolism and cardiac mitochondrial structure and function. Male 8-week-old BALB/c mice were exposed to urban air (UA, 27±8 µg PM2.5/m3) or filtered air (FA, 2±1 µg PM2.5/m3) in whole-body exposure chambers for up to 16 weeks. After 12 weeks, lung inflammatory cell recruitment was evidenced by histology in UA-exposed mice. Interestingly, impaired redox metabolism, characterized by increased lung GSSG content, decreased SOD activity, and increased NOX activation, preceded local inflammation in UA-exposed mice. Moreover, PM2.5 uptake and enhanced nitric oxide production was observed in alveolar macrophages from UA-exposed mice by electron microscopy and flow cytometry, respectively, together with increased proinflammatory cytokine levels (TNF-α and IL-6) in bronchoalveolar lavage and plasma. In the heart of UA-exposed mice, impaired tissue oxygen metabolism and altered mitochondrial ultrastructure and function were observed, by decreased active state respiration by 48%, inner membrane depolarization, decreased ATP production by 17%, and enhanced H2O2 release by 39%. This scenario led to a significant increase in infarct size following in vivo myocardial ischemia/reperfusion injury, from 43±3% of the area at risk in FA-exposed mice to 66±4% in UA-exposed mice ($p<0.01$). Taken together, our data unravel some of the pathways that might explain the adverse health effects of air pollution exposure, and ultimately highlights the importance of considering environmental factors in the development of cardiovascular diseases.

OP24

NETs formation in blood of psoriatic patients modified by cannabidiol

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Psoriasis development is associated with increased reactive oxygen species generation what leads to oxidative stress in patients' skin, as well as blood cells, including neutrophils. As antioxidants can provide protection, the aim of this study was to evaluate the effects of cannabidiol (CBD) on neutrophil extracellular trap (NET) formation in psoriatic and healthy neutrophils.

Important markers of NETosis were measured in healthy and psoriatic neutrophils after incubation with CBD, lipopoly-saccharide (LPS), and LPS + CBD). The percentage of neutrophils undergoing NETosis and the level of NETosis markers (cfDNA, MPO, elastase) were higher in the neutrophils and blood plasma of psoriatic patients, compared to controls. After LPS treatment, all of the markers of NETosis, except elastase, and p47 and citrullinated histones, were increased in samples from healthy subjects and psoriasis patients. CBD reduced also the concentrations of NETosis markers, which was more pronounced in psoriatic neutrophils and neutrophils treated with LPS in both psoriatic and healthy participants. These results suggest that psoriatic patients' neutrophils are at a higher risk of NETosis both in vitro and in vivo.

CBD reduces NETosis, mainly in psoriatic neutrophils, possibly due to its antioxidant properties. The anti-NET properties of CBD suggest the positive effect of CBD in the treatment of autoimmune diseases.
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OP25
Direct antiviral agents improve systemic redox balance in patients affected by chronic hepatitis C

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Introduction: Oxidative stress is a process deeply involved in the progression of liver damage during chronic hepatitis C. The management of patients infected by chronic hepatitis C has recently advanced owing to the effectiveness and safety of direct-acting antivirals (DAAs). This study investigated the impact of DAA treatment on circulating markers of oxidative stress and antioxidant defence in a cohort of patients affected by chronic hepatitis C.
Methods: An observational study on 196 patients who were treated with DAAs for HCV-related hepatitis was performed. Patients were assessed at baseline, 4 weeks after the initiation of therapy (4wks), at the end of treatment (EoT), and 12 weeks after the EoT (SVR12). Circulating oxidative stress was determined by measuring serum hydroxynonenal (HNE) and malondialdehyde (MDA)-protein adducts, and 8-hydroxydeoxyguanosine (8-OHdG). Antioxidant status was evaluated by measuring the enzymatic activity and mRNA expression of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in peripheral blood mononuclear cells.
Results: We observed a reduction of serum 8-OHdG at 4wks, while the circulating level of both HNE and MDA-protein adducts diminished at EoT; all these markers persisted low at SVR12. On the other side, we reported an increase in the enzymatic activity of all the antioxidant enzymes in PBMC at EoT and SVR12. Taking into account circulating 8-OHdG and antioxidant enzyme activities, patients with high fibrosis stage were those that had the most benefit from DAA therapy.
Discussion: This study indicates that treatment with DAAs improves the circulating redox status of patients affected by chronic hepatitis C. This positive impact of DAA therapy may be related to its effectiveness on cutting down viremia and pro-inflammatory markers. The improvement in circulating redox balance may be effective in the prevention of morbidity related to the oxidative stress caused by HCV infection.
Keywords: Oxidative stress, hepatitis C, direct acting antivirals (DAAs)

OP26
The NADPH oxidase NOX4 regulates metabolism in hepatocellular carcinoma cells

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The NADPH oxidase (NOX) family has emerged in the last years as an important source of reactive oxygen species (ROS) in signal transduction. The isoform NOX4 has been implicated in a variety of physiological and pathological processes. In recent works, we found that stable knockdown of NOX4 expression in liver tumor cells increases their proliferative capacity in vitro and enhances their tumorigenic potential in xenografts mice, resulting in earlier onset of tumor formation and increase in tumor size. NOX4 could also regulate other cellular processes that occur later in progression and that favor tumor metastasis, such as migration and invasion. NOX4 gene deletions are frequent in HCC patients, correlating with higher tumour grade. Here we aim to determine the molecular mechanisms regulated by NOX4 in liver cells that could explain its tumor suppressor functions. A proteomic analysis, by comparing HCC control cells with cells where NOX4 had been silenced or overexpressed, allowed the identification of metabolism as one of the highest affected processes. Silencing NOX4 in PLC/PRF/5 cells increased both the glycolysis and oxidative phosphorylation pathways, while the overexpression of NOX4 in SNU449 cells showed opposite effects. A detailed transcriptomic and metabolomic

analysis indicated that NOX4 could be regulating fatty acid metabolism. We found differences in gene expression related to fatty acid transport, oxidation and de novo synthesis, as well in the amount of monoacylglycerol, diacylglycerol and carnitine intermediates, which indicate an inverse correlation between the expression of NOX4 and the cell capacity to use fatty acids. Silencing NOX4 induced changes in the amount and dynamics of the mitochondria, increase in the protein levels of complex IV and V and higher ATP levels. Our data indicate that NOX4 regulates c-Myc, which could mediate the changes observed. Importantly, the catalytic activity of NOX4 was required for some of the effects observed.

OP27 Hexameric procyanidins inhibit colorectal cancer cell growth through redox regulation of the epidermal growth factor signaling pathway

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Dietary proanthocyanidins (PAC) consumption is associated with a decreased risk for colorectal cancer (CRC). Dysregulation of the epidermal growth factor (EGF) receptor (EGFR) signaling pathway is frequent in CRC. We previously showed that hexameric PAC (Hex) exert anti-proliferative and pro-apoptotic actions in human CRC cells. Redox-regulated mechanisms contribute to enhance and prolong EGFR activation. This work investigated if Hex could exert anti-CRC effects in part through its capacity to affect the redox regulation of the EGFR pathway. A transient NADPH oxidase (NOX) activation occurs upon binding of EGF to the EGFR, which enhances/prolongs the activation of the pathway. We observed that, in proliferating Caco-2 cells, EGF triggered a transient ROS increase (evaluated with DHDCF, DHE and Amplex red). This was mitigated by Hex and by three NOX inhibitors (DPI, apocynin and Vas-2870). Hex, DPI, apocynin and Vas-2870 also inhibited EGFR phosphorylation at Tyr1068 and the downstream activation of pro-proliferative and anti-apoptotic signaling cascades, i.e. Raf/MEK/ERK1/2 and PI3K/Akt. Hex also inhibited other events in the EGFR pathway, including receptor dimerization and internalization. Importantly, Hex acted synergistically with the EGFR-targeted chemotherapeutic drug Erlotinib, both in their capacity to decrease EGFR phosphorylation and inhibit cell growth. Thus, dietary PAC could exert anti-CRC actions by modulating, through redox- and non-redox regulated mechanisms, the EGFR pro-oncogenic signaling pathway. Additionally, Hex could also potentiate the actions of EGFR-targeted drugs.
Keywords: proanthocyanidins; NADPH oxidase; epidermal growth factor receptor; colorectal cancer; redox regulation

OP28
Investigating the stress sensitivity of the secretory pathway in cancer cells using high-resolution fluorescence imaging

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Cancer cells have a very efficient secretory pathway and numerous molecules trafficking through this pathway underpin high proliferation rates and other cancer-specific hallmarks. The endoplasmic reticulum (ER) is the first organelle of the classical secretory pathway and the primary site for crucial protein folding and modification processes including redox reactions.
We recently established live-cell imaging of fluorescent protein (FP) tagged cargos during their journey from the ER to the Golgi apparatus and further to the plasma membrane or extracellular space. The high-resolution fluorescence time-lapse imaging of fluorescent transmembrane and luminal cargo proteins in HeLa cells allows the investigation of the stress-sensitivity of ER-to-Golgi transport in this cancer cell model. Interestingly, our data revealed that ER-to-Golgi transport remains highly efficient under energy stress despite severe reductions in subcellular ATP levels. Treatment with the antimetabolite 2-deoxy-D-glucose (2-DG), on the other hand, completely abolished secretory transport of the fluorescent cargo constructs (1).
So far, the potential of our experimental framework has been tested for energy and Ca^{2+} stresses yielding new insights into the vulnerability of the secretory pathway in cancer cells. Our protocols open the door for further experiments to investigate how free radicals and other reactive molecules might interfere with the secretory activity of interesting cell models.

Keywords: ER-to-Golgi transport; vesicle trafficking; secretory pathway; cancer cell metabolism; fluorescent protein technology; live-cell imaging; protein transport and sorting; stress sensitivity; oxidative stress
References:
(1) Rauter, T., Burgstaller, S., Gottschalk, B., Ramadani-Muja, J., Bischof, H., Hay, J. C., Graier, W. F. & Malli, R. (2020). ER-to-Golgi Transport in HeLa Cells Displays High Resilience to Ca^{2+} and Energy Stresses. Cells, 9(10), 2311.

ABSTRACTS of SFRR-INTERNATIONAL 2021 VIRTUAL MEETING

Narrated Communications

NC1
The cellular vimentin network undergoes distinct reorganizations in response to diverse electrophiles or mutations of its single cysteine residue

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Vimentin is a type III intermediate filament protein which plays key roles in essential cellular processes such as cell migration, division, organelle positioning and homeostasis (Duarte, 2019). Moreover, vimentin behaves as a stress sensor. We previously reported that the single cysteine residue of vimentin (C328) is the target for modification by oxidants and electrophiles, such as 4-hydroxynonenal, diamide or 15-deoxy-PGJ_2. These compounds induce marked alterations in filament assembly *in vitro* and in vimentin network reorganization in cells. The attenuation of these effects in a C328S vimentin mutant supports the importance of this residue in the transduction of oxidative or electrophilic modifications into cytoskeletal responses (Mónico, 2019).
Here we have used vimentin-negative SW13/cl.2 cells transfected with GFP-vimentin constructs in order to explore the structure-function relationships of C328 modification. We have observed that treatment of cells with several oxidants and electrophiles, including 4-hydroxynonenal, 1,4-dinitroimidazole or H_2O_2, disrupts the organization of GFP-vimentin structures leading to diverse patterns depending on the agent used. Nevertheless, these agents can modify multiple cellular targets. To directly address whether structural modifications at C328 play a role in vimentin reorganization we have studied the behaviour of several mutants at this site. We have found that different mutations provoke the assembly of GFP-vimentin constructs into morphologically distinct arrays in two vimentin-deficient cell lines, i.e. SW13/cl.2 and MCF7 cells. Interestingly, a C328H mutant forms robust structures that are more resistant to disruption by oxidants and electrophiles than the wild type.
Altogether these observations strengthen the role of vimentin C328 as a key stress sensor and suggest that structurally different modifications at this site could result in morphologically and/or functionally distinct reorganizations of the intermediate filament network.
Duarte (2019) *Nat Commun*, 10:4200.
Mónico (2019) *Redox Biol* 23:101098.
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NC2
oxSWATH applied to the study of the alteration of intracellular and extracellular proteome of cells in response to oxidative stress

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In the present work, an exhaustive characterization of the proteome adaptions to oxidative stress was performed using the oxSWATH[1] approach. This method allows integrating the information regarding relative cysteine oxidation with the analysis of the total protein level. Thus, in a single analysis, it was possible to evaluate the alteration considering the redox status of the proteins and performed a generic differential proteomics analysis of the cells exposed to an acute stimulation with hydrogen peroxide. To completely characterize the cellular response, both the cells and the secretome were analyzed, covering the intracellular and extracellular responses, respectively. A total of 915 proteins were altered upon oxidative stress, from which, 90 were altered in both intra- and extracellular space. Moreover, a clear tendency for a remodeling of the extracellular space was observed, with near 80% of the altered proteins found altered in the secretome. The analysis of the overall redox status of the proteins reveals a tendency to have a reduced environment in the extracellular space, while an equilibrium between the reduced and oxidized proteins is achieved in the intracellular environment. Again, a higher number of secreted proteins present an alteration of their redox status upon oxidative stress when compared with the intracellular proteins (250 and 61 proteins, respectively). From those, only 4 were commonly altered between the two

cellular spaces. Overall, these results indicate that there is a differential adaptation of the intracellular and extracellular proteomes, with the extracellular space being particularly affected by oxidative stress. Moreover, the potential of the oxSWATH method was confirmed in this work since a truly comprehensive evaluation of proteomics changes upon the oxidative stimulus was achieved using a single approach.
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NC3

H_2O_2 biosensors HyPer2, HyPer3 and GFP2-Orp1 detect rapid pH changes due to environmental CO2 fluctuations, in addition to intracellular H_2O_2, in isolated skeletal muscle fibres

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Hydrogen peroxide (H_2O_2) is one of the Reactive Oxygen Species (ROS) that seems to play an essential role in pathophysiological processes. H_2O_2 might act as a signaling molecule and modulate different crucial cellular signaling pathways, such as the glucose uptake in skeletal muscle, where H_2O_2 has been proposed to play an important role.
HyPer2, HyPer3 and GFP2-Orp1 are hydrogen peroxide biosensors. We use these biosensors to monitor intracellular H_2O_2 in single skeletal muscle fibres isolated from the flexor digitorum brevis (FDB) mouse muscle. Previously, the coding sequences of these biosensors were microinjected and electroporated in FDB. Isolated fibres in culture that expressed one of the biosensors were settled incubation chamber coupled to the fluorescence microscope. The chamber maintains temperature (37ºC), environmental CO_2 (5%) and humidity. Different time course experimental conditions were performed where fibres were exposed to different agents (insulin, interleukin 1β, H_2O_2, DTT) and intracellular H_2O_2 flux was registered in real time using fluorescence microscopy imaging analysis. We observed that when there were environmental CO_2 (5%) fluctuations, due to initial medium stabilization or occasional interruption of CO_2 supply, the biosensors showed changes in the fluorescence emission, which were registered. The main consequence of CO_2 fluctuations is the change in the pH of medium. The main part of the biosensor structure is a fluorescent protein, YFP in de case of HyPer2 and HyPer3, and GFP2 in GFP2-Orp1. It has been reported that these fluorescent proteins are sensitive to pH and this might be a disadvantage for the biosensors. However, we believe that this pH sensitivity should be considered as an additional property of this biosensors, since they provide information in real time about the rapid changes of pH due to environmental fluctuation of CO_2 and likely other gases such as O_2 or N_2.
Keywords: Hydrogen peroxide; CO2; biosensors; skeletal muscle fibres

NC4

PRDX2- and PRDX3-rsGFP2 fusions: response to relevant oxidants and redox sensors during hypoxia and reoxygenation inside living cells

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Peroxiredoxins (Prx) are thiol-dependent peroxidases playing key roles in antioxidant defence and redox signalling and are preferential intracellular targets for peroxides. Genetically encoded probes based on Prx and redox-sensitive GPF2 (rsGFP2) fusions allow to detect elevated H_2O_2 levels in stimulated or stressed cells. We have developed bovine aortic endothelial cells (BAECs) stably expressing a hsPrx2-rsGFP2 protein and evaluated the response to exogenous addition of H_2O_2. The oxidation levels of the probe (OxD) were dependent on peroxide concentration but did not reach maximum levels, probably due to partial overoxidation of Prx, as detected by western blot. Interestingly, the endogenous and rsGFP fusion forms of Prx2 showed distinct sensitivity to overoxidation. We also measured the real-time oxidation of hsPrx2-rsGFP2 sensor when the cells were exposed to a continuous flux of H_2O_2, peroxynitrite and drugs known to induce ferroptosis via accumulation of lipid peroxides (RSL3 and erastin). Our group is advocated to the study of redox responses to hypoxia and reoxygenation. We observed a time dependent oxidation of the peroxide probe 2',7'-dichlorofluorescein (***DCF***) during reoxygenation after 2 hours of hypoxia, which was accompanied by a reversible oxidation of endogenous Prx1 and 2 to disulphide-bond dimers. Accordingly, oxidation of hsPrx2-rsGFP2 was detected under the same conditions, making this probe an excellent tool for our studies in the living cell. A *hs*Prx3-rsGFP2 containing the Prx3 mitochondrial

target was developed. Confocal microscopy studies confirmed the localization of the probe in the mitochondria. We aim to use both probes to study the redox response of the cell to hypoxia and reoxygenation in both cellular compartments.

NC5
Performance evaluation tests of an auto-fluorescence observation system for non-invasive biological measurements

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Conventional oxidative stress markers, such as lipid-derivers free radicals have been studied to cause damages to cell membranes, proteins and other biomolecules. Decompositions of lipid hydroperoxides are known to release excited triplet states of biomolecules composed with carbonyl groups. The previous study suggested that ultra-weak photon emissions of the carbonyl groups composed with various wave lengths. On the other hand, more detailed investigations on *in vivo* redox status are needed to elucidate the mechanisms contributing to damage caused by stress. Recently, glycative stress related to accumulation of advanced glycation products (AGEs) might be important to monitor in the redox status because AGEs have been used as biomarkers for non-invasive measurement techniques. However, a skin condition marked by an overgrowth of layers of horny skin and distance from skin surface to high moisture stage layer might affect measuring auto-fluorescence *in vivo*. To elucidate mechanisms of photon emissions from human fingers including fluorescent oxidation products, we executed performance tests of an auto-fluorescence observation system. Our findings provide that auto-fluorescence intensities of human fingers changed during COVID-19 related crisis. The auto-fluorescence observation system for non-invasive biological measurements might be a lifestyle habit improvement support device.

NC6
Redox control of the transcriptional circadian rhythmicity by SOD2

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Endogenous control of circadian rhythmicity is mainly governed by clock genes at the genomic level. Furthermore, both clock genes and redox regulation also modulate cell metabolism, thus showing a closed and strong interconnection between both regulatory pathways.
Mitochondrial superoxide dismutase (SOD2/MnSOD) is a key antioxidant and redox-regulating enzyme, depurating superoxide anion mainly generated in the electron transport chain. The increase in the activity of this enzyme has been postulated as a major event during the antioxidant defense response; on the other hand, loss of SOD2 function constitutes a significant oxidative stress factor. With this premise, the work presented here used two transgenic murine models for SOD2 characterized by either a reduced function in hemizygous ($SOD2^{+/-}$) and by a SOD2 overexpressing mice ($SOD2^{+/++}$) in order to study the role of oxidative stress and redox regulation on the physiological circadian transcriptional rhythmicity.
Interestingly, both transgenic models, $SOD2^{+/-}$ and $SOD2^{+/++}$, displayed a similar transcriptional profile which differed to WT, sharing some transcriptional changes regarding cytokeratin, calcium binding, and kallikrein serine proteases. Both genotypes presented a significant loss of rhythmic metabolic transcripts, when compared to WT, resulting in a lower number of rhythmic transcripts. However, the changes in circadian rhythmic transcripts in heterozygous $SOD2^{+/-}$ mice were greater than those observed in $SOD2^{+/++}$ overexpressing mice. Therefore, a change in the expression pattern of ARNTL/BMAL1, the transcriptional decrease in NADH dehydrogenase or the pro-inflammatory transcript profile were among the major features observed in $SOD2^{+/-}$ mice.
The study points that deregulation of SOD2 expression accounts for important changes in the metabolic transcriptional machinery, especially in a situation of oxidative stress, in which clock genes and the metabolic activity appear to be highly compromised.
Keywords**:** Superoxide dismutase 2, Circadian rhythm, Metabolism, ARNTL, Electron transport chain.
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NC7
Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease

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Free radicals and oxidants are involved in physiological signaling pathways, although an imbalance between pro-oxidant and anti-oxidant systems in favor of the former leads to major biomolecular damage. This is the so-called oxidative stress, a complex process that affects us all and is responsible for the development of many diseases. Lipids are very sensitive to oxidant attack and to-date, malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) and F2-isoprostane are the main biomarkers for lipid peroxidation assessment. They all derive from polyunsaturated fatty acids (PUFAs) either by enzyme-catalyzed reactions (physiological) or by non-enzyme reactions (pathological). Lipid peroxidation increases in several pathological and physiological situations. Over the years, in the laboratory, we have determined MDA as an index of lipid peroxidation in human plasma samples in different physiological situations as well as in disease. According to our results, we suggest the following MDA values determined by HPLC as reference values in Aging: Young 0.85 mM; Adult 1.25 mM; Old 2.54 mM – Frailty: Old Non-frail 2.19 mM; Old Pre-frail 2.80 mM; Old Frail 3.63 mM – Chronic obstructive pulmonary disease: 0.55 mM – Diabetes mellitus: 1.61 mM – Liver cirrhosis: 3.51 mM – Hemodialysis: 5.00 mM – Alzheimer's disease: 7.40 mM. In conclusion, reliable measurement of MDA is challenging. Special emphasis should be made on sample collection, blood coagulation and the original PUFA composition of the sample. So far, HPLC has proven to be specific and more sensitive for both, free and adducted MDA. Thus, MDA is a valuable biomarker for the assessment of lipid peroxidation in human plasma under different physiological and pathological situations.
Keywords: MDA, reference values, lipid peroxidation, HPLC, oxidative stress.

NC8
Diffusion and Removal Kinetics of Hydrogen Peroxide in Brain Tissue

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Hydrogen peroxide is a major biological oxidant which plays roles in redox regulation of biological functions over a range of concentrations, from low (1-10) nM, encompassing redox signaling under physiological conditions, to the activation of adaptative cell responses and, at one order of magnitude higher concentration, to damage of biomolecules. Its concentration is maintained through tight regulation by activity-dependent production and efficient removal systems including catalase, glutathione peroxidase and peroxiredoxin systems in different compartments. Diffusion and removal kinetics of H_2O_2 in the brain is largely unknown.
Here we used ruthenium-purple modified carbon fiber electrodes to study the concentration dynamics of exogenously applied H_2O_2, focusing on diffusion and modulation of removal in striatum slices and *in vivo*.
In brain slices diffusion of H_2O_2 followed a 1st order exponential decay function, with a diffusion coefficient of D=2.8 x 10^{-6} cm^2 s^{-1} and $t_{1/2}$ = 2.5 ± 0.9 s. Inhibition of catalase did not change the decay kinetics of H_2O_2, while inhibition of glutathione peroxidase and peroxiredoxin increased the $t_{1/2}$ of H_2O_2 in striatal slices to 3.8 ± 1.2 s and 4.0 ± 0.9 s, respectively. Metabolic poisoning (no glucose and CN^-) significantly increased $t_{1/2}$ to 5.8 ± 1.2 s, a value similar to that found in a 0.2% agarose slice ($t_{1/2}$ = 6.5 ± 0.8 s). *In vivo* studies in the striatum of anesthetized rats revealed similar diffusion profile, with $t_{1/2}$ = 1.3 ± 0.6 s, which increased to 4.3 ± 2.2 s following cardiac arrest.
These results reveal quantitative kinetic parameters regarding the dynamics of H_2O_2 as a diffusible intercellular brain messenger. In the absence of circulating red blood cells (RBC), glutathione peroxidase and peroxiredoxins are the main contributors towards H_2O_2 removal in brain tissue. *In vivo* data suggests that catalase in RBC may contribute to shaping extracellular H_2O_2 concentration profile.
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NC9
How far can hydrogen peroxide travel in blood circulation?

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In response to a mechanical or other type of stimuli, vascular endothelial cells release superoxide to the extracellular medium. Through a dismutation reaction, part of this secreted superoxide is readily converted into hydrogen peroxide, which can act as an autocrine and/or a paracrine signalling agent. In this work we developed a computer simulation to quantify the restrictions of hydrogen peroxide signalling in capillaries and arterioles. This computer simulation considered the dismutation reaction, as well as the superoxide/hydrogen peroxide release/uptake, diffusion and transport by the blood flow. For plausible cellular rates of superoxide production, *local* hydrogen peroxide concentrations in blood plasma may reach ~0.1 μM. Maximal concentrations occur within 10 μm and 500μm of the start of the superoxide production domains, in capillaries and arterioles, respectively.
We conclude that (i) signalling through superoxide/hydrogen peroxide release to the circulation can only be autocrine in the case of the capillaries and may be paracrine in arterioles; (ii) hypothetical signalling mechanisms must be sensitive to sub-μM extracellular hydrogen peroxide concentrations, which requires peroxiredoxins or peroxidases acting as hydrogen peroxide receptors. The poster also addresses signalling at the basal side of the endothelium.

NC10
Effect of longitudinal magnetic field to the linear particle-beam track on yields of hydroxyl radical and hydrogen peroxide in water

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The effect of a magnetic field loaded parallel to the ion-beam track on the generations of reactive oxygen species (ROS) in water was investigated. It was reported that loading a magnetic field parallel to the particle beam track exaggerates the biological effects, i.e. cell lethality, of the particle beam. However, the mechanism of the enhanced cell death delivered by a logigudinal magnetic field to the beam track has not been clarified yet.
Local density of •OH generation was estimated by a method based on EPR spin-trapping technique. A series of reaction mixtures containing varying concentrations (0.76–2278 mM) of DMPO was prepared, and transferred to a polyethylene bag for irradiation. Then, the samples were irradiated 16 Gy of carbon- or iron-beam at the Heavy-Ion Medical Accelerator in Chiba (HIMAC, NIRS/QST, Chiba, Japan) with or without longitudinal magnetic field (0.0, 0.3, or 0.6 T).
O_2-dependent and O_2-independent H_2O_2 yield was measured. An aliquot of ultra-pure water was irradiated by carbon-ion beam with or without longitudinal magnetic field. Irradiation experiments were performed under air or hypoxic (<0.5% oxygen) conditions. H_2O_2 generations in irradiated water samples were quantified by an EPR spin-trapping method, which measures •OH synthesized from H_2O_2 by the UVB irradiation.
Relatively sparse •OH generations caused by particle beams in waster were not affected with loading magnetic field on beam track. Oxygen dependent H_2O_2 generation was decreased and oxygen-independent H_2O_2 generation was increased under loading magnetic field parallel to the beam track. Loading magnetic field parallel to the beam track could make •OH generation denser, or could make reaction of dense •OH increased.

NC11
High concentrations of caseins in milk propitiate the occurrence of chain reactions, and propagation of oxidative damage

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Milk is one of the most important and valuable agricultural commodities worldwide, as a consequence of its low price, widespread consumption and high nutritional value. However, high temperatures, pressures, and light exposure encountered during processing, results in exposure of milk components to oxidative stress. Caseins represent 80% of the total protein content in milk, and due to their amphiphilic nature, they stabilize the oil-water interface; this results in exposure to both hydrophilic and lipophilic oxidants. We hypothesized that exposure of caseins (α-, β-, and κ-casein) to light in the presence of riboflavin (RF, vitamin B2; an endogenous photosensitizer found in milk), or peroxyl radicals (ROO•) trigger modifications to the amino acid side-chains of caseins, with downstream consequences for structure and function. We

also predict that the high casein concentrations in milk, and their micellar structure, would facilitate chain reactions and damage propagation. The occurrence and yield of modifications was analyzed in control and oxidized samples at low (1 mg/mL) and high (up to 27 mg/mL) casein concentrations by SDS-PAGE, dynamic light scattering, liquid chromatography-mass spectrometry and transmission electron microscopy. Our results demonstrate that both ROO• and RF-mediated oxidation generate multiple different oxidation products (from Trp, Tyr, Met, His, Lys, Cys) and crosslinks including diTyr (for multiple caseins) and disulfide bonds (with κ-casein). At low casein concentrations (1 mg/mL) higher yields of crosslink products were detected by SDS-PAGE. However, at high protein concentrations (10–27 mg/mL) a great extent of total amino acid consumption and modification were detected, consistent with complex oxidation mechanisms and potential chain reactions. These results highlight the importance of understanding, at a molecular level, the processes occurring during oxidation of proteins in complex biological matrices such as foodstuffs, to enable the development of new strategies to improve industrial processing methods.

NC12
Modulation of mitochondrial metabolism markers and mitochondrial morphology by aging and CYB5R3 overexpression in transgenic mice

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Aging is defined as a gradual decline of the normal physiological functions of an organism and is considered as the most important risk factor for most chronic diseases. Nowadays, there is great interest in identifying enzymes which promote healthy aging and increase longevity. Cytochrome b_5 reductase-3 (CYB5R3), which catalyzes electron transfer from NADH to cytochrome b_5 and also to alternative electron acceptors as plasma membrane coenzyme Q or several exogenous compounds, increases longevity, improves mitochondrial function, decreases oxidative damage and protects against induced cancer in transgenic mice. We have hypothesized that mitochondrial efficiency is maximized in CYB5R3-transgenic mice which leads to improved energy production with less ROS generation and better mitochondrial preservation with aging. To elucidate how CYB5R3 overexpression extends longevity, we have focused our efforts towards the study of mitochondrial metabolism and morphology in hindlimb skeletal muscle, as a model of a postmitotic tissue which plays a relevant role in aging, from young and old mice of wild-type and CYB5R3-overexpressing genotypes. CYB5R3 was highly overexpressed in skeletal muscle, indicating that this tissue is a suitable model to study the direct effects of CYB5R3 overexpression in the cellular physiology. CYB5R3 levels were not affected by aging in skeletal muscle. CYB5R3 overexpression increased the levels of mitochondrial complexes particularly in old mice, which could be related with the prevention of mitochondrial dysfunction associated with aging. Our electron microscopy study revealed that mitochondrial size was decreased with aging, but also in mice overexpressing CYB5R3. However, CYB5R3 overexpression strongly attenuated the changes of mitochondrial size with aging. Optimization of mitochondrial physiology thus emerge as a key mechanism related with the antiaging effect of CYB5R3 overexpression. Moreover, our data suggest that therapies aimed on increasing CYB5R3 levels or activity might be feasible antiaging approaches at any age.
Keywords: Cytochrome b_5 reductase, Aging, Mitochondrial metabolism, Longevity, Skeletal muscle.

NC13
Regulation of coenzyme Q biosynthesis by n-3 polyunsaturated fatty acids

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Dietary fats are both a source of energy and essential components in diets. Most of them can be synthesized by the cells, but the omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) must be ingested through the diet to avoid deleterious effects on health. The main source of lipids in the diet influences the phospholipid composition of cellular membranes and cam produce changes in the molecular and metabolic activities of the cells, especially in mitochondrial membranes. Coenzyme Q (CoQ) is the only lipid-soluble antioxidant that animal cells can synthesize. Dietary supplementation with CoQ has shown promising effects to treat mitochondrial disorders related with CoQ deficiency and as an anti-aging therapy. However, its low absorption and bioavailability has questioned the effectiveness of supplementation strategies with this antioxidant, which has prompted a search for interventions able to induce endogenous CoQ biosynthesis. In our study we focused in gaining a deeper knowledge into the relation between specific fat dietary intake, particularly n-3 PUFAs,

and CoQ biosynthesis using both in vivo and in vitro models. We found that dietary fats exert differential actions on CoQ biosynthesis, and specifically n-3 PUFAs target directly this pathway and increase hepatic CoQ content. Furthermore, n-3 PUFAs are be able to alter the proportion between CoQ isoforms, inducing the biosynthesis of CoQ10 over CoQ9 through inhibition of farnesyl diphosphate synthase (FDPS), which can be recapitulated by genetic silencing of FDPS and by its pharmacological inhibition with zoledronic acid. Our results uncover n-3 PUFAs as novel modulators of CoQ biosynthesis in mammals. Furthermore, we identified for the first time zoledronic acid as drug that targets CoQ biosynthesis, which must be taken into account to fully understand its pharmacological effects.
Keywords: Coenzyme Q, dietary fats, n-3 PUFAs, zoledronic acid
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NC14
Low abundance of NDUFV2 subunit of the hydrophilic complex I domain predicts animal longevity

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Mitochondrial free radical production, specifically at complex I (Cx I), has been suggested to be one of the determinants of species longevity. The present study is designed as a comparative approach to analyze the Cx I subunits in heart tissue from 8 mammalian species with a longevity ranging from 3.5 to 46 years. Gene expression and protein content of selected Cx I subunits were analyzed using droplet digital PCR and western blot, respectively. Our results demonstrate: 1) the existence of species-specific differences in gene expression and protein content of Cx I in relation to longevity; and 2) that the achievement of a longevity phenotype is associated with low protein abundance of subunit NDUFV2 from the matrix hydrophilic domain of Cx I. The N1a Fe-S cluster contained in NDUFV2 does not take part in the electron transfer path of the peripheral arm of complex I, and it has been suggested that can regulate free radical generation. The lowered NUDFV2 subunit abundance present in long-lived animal species suggests that this subunit can modulate mitochondrial free radical generation, limiting its production and probably explaining the lower amount of this free radical species generated in long-lived animal species. Therefore, it is suggested that the NDUFV2 subunit content modulates basal free radical production in each animal species in agreement to its longevity and would explain the physiological significance of the off-path location of N1a cluster.

NC15
Succination of protein thiols in human frontal cortex during aging

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Human brain evolution toward complexity has been achieved by increasing energy supply as the main adaptation in brain metabolism. Energy metabolism, like other biochemical reactions in aerobic cells, is under enzymatic control and is strictly regulated. Nevertheless, physiologically unavoidable, uncontrolled and deleterious reactions take place. It has been proposed that these deleterious reactions constitute the basic molecular mechanisms that underlie the maintenance or the loss-of-function of neurons and cerebral functions during brain aging. In this work, we focus our attention on the role of the nonenzymatic and irreversible adduction of fumarate to the protein thiols, which leads to the formation of S-(2-succino)cysteine (2SC), a process called protein succination, and considered a marker of mitochondrial stress. For this purpose, 2SC was detected and quantified in the frontal cortex (FC) of healthy humans covering an age range from 40 to 90 years old using mass spectrometry techniques. Our results demonstrate i) the unambiguous formation of 2SC in human brain, ii) that the steady-state level of 2SC in FC is lower compared to other brain areas, and iii) in contrast to other non-enzymatic posttranslational modifications, 2SC does not increases with age. Our findings suggest a better protection of cysteine residues and their properties by specific and reversible mechanisms such as S-sulfhydration and polysulfidation to prevent cysteine irreversible modification, and suggest that in human FC mitochondrial metabolic stress is strictly sustained within physiological limits throughout the healthy adult lifespan, being a key mechanism in supporting neuronal function and survival.
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NC16
Extracellular Vesicle Production by Skeletal Muscle: Role in Neuromuscular Ageing

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Generation of reactive oxygen species is increased in muscle following contractions and this results in the activation of redox-responsive transcription factors including Heat Shock Transcription Factor-1 (HSF-1), and subsequent increased Heat Shock Protein (HSP) production. This robust stress response is in contrast to neuronal cells which are proposed to be unable to mount a stress response. Exosomal HSP transfer maintains proteostasis in recipient cells and we hypothesise that muscle supports peripheral neurons by exosomal protein transfer during contractions. HSP generation by muscle following contractions is attenuated in old mammals, potentially due to altered contraction-induced ROS production. We further hypothesise that this will result in altered Extracellular Vesicle (EV) transfer of cytoprotective proteins and failure to maintain proteostasis in neurons, resulting in neuronal degeneration.
Flexor digitorum bravis (FDB) muscle fibers were isolated from adult (6-8m) and old (24-26m) mice and contracted using electrical stimulation. Media was collected from quiescent and contracted fibers, EVs purified and characterised via NanoSight analysis for size/number and western blotting for HSP content.
Preliminary data suggest that the quantity of EVs released from quiescent fibers from adult and old mice were similar and contractions resulted in an increased number of EVs being released compared with quiescent fibers. The size distribution of the EVs from fibers from old mice was altered compared with adult mice. Additionally, the total protein content of EVs was significantly lower in those from old mice compared with both quiescent and contracted fibers from adult mice. Content of HSPs was increased in EVs from adult mice fibers following contractions whereas data suggest the HSP60 content of EVs produced by old quiescent fibers was elevated, with minimal increase following contractions. Additional data will examine the protein content of EVs using proteomics and examine uptake of EVs by neuronal cells.
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NC17
Development of age-related loss of muscle mass and function – role of oxidative DNA damage repair systems

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An increased presence of the oxidised DNA base lesion seen in muscles of old mice is 8-oxo-7, 8-dihydroguanine (8-oxoG). 8-oxoG is repaired by the 8-oxoguanine DNA glycosylase-1 (OGG1)-initiated DNA base excision repair pathway. This is proposed to lead to activation of NF-kB. Skeletal muscle of old mice demonstrated a chronic activation of NF-κB but the mechanisms by which this occurs are unclear. We hypothesised that chronic oxidative DNA damage leads to the chronic increase in activation of NF-kB by OGG1 and the subsequent production of inflammatory cytokines by skeletal muscle, contributing to age-related deterioration of muscle function.
An in vitro model of oxidative DNA damage was established by treating C2C12 myoblasts with H_2O_2 (10μM-1000μM). DNA damage repair responses including OGG1, poly(ADP-ribose) polymerase-1 (PARP-1), cleaved-PARP-1, NF-kB activation and pro-inflammatory gene expression and release by cells were determined. The effect of TH5487 (5μM), a selective OGG1 inhibitor on NF-kB activation and cytokine production was also determined.
Cell viability was maintained in myoblasts treated with 50μM H_2O_2 but Ogg1 mRNA levels were significantly increased as was acetylation of OGG1, PARP-1 and cleaved PARP-1 protein levels and activation of NF-κB with an increased expression of a number of pro-inflammatory cytokines. The H_2O_2-mediated increase in NF-kB DNA binding activity was significantly decreased when cells were pretreated with TH5487. Experiments in isolated viable muscle fibres from adult mice has demonstrated an increase in NF-κB activation following treatment with 50μM H_2O_2. Proteomic and mRNA analyses of muscles from adult and old mice are being examined for evidence of changes in DNA repair pathways with age and a computational model (Copasi) of DNA damage repair is currently being designed to identify druggable target(s) to modify activation of NF-κB in muscles of old mice.

NC18
Redox proteomics reveal differential effects of both ageing and exercise on human skeletal muscle

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Reactive oxygen species (ROS) are recognised as important signalling molecules in healthy skeletal muscle. Redox sensitive proteins can respond to intracellular changes in ROS via reactive thiol groups on Cysteine (Cys) residues. While exercise is known to induce the generation of ROS that results in the activation of a number of transcription factors, it has been suggested that ageing attenuates these redox regulated adaptive responses to acute exercise.
In the present study, we have applied a redox proteomic approach to study the vastus lateralis muscles of young (n = 6 male, 6 female; 18-30 yrs) and older (n = 6 male, 6 female; 64-79 yrs) adults. Participants completed a high intensity cycling exercise bout consisting of five sets of two-minute intervals performed at maximal aerobic power output. Samples were collected in the resting state, and immediately following the first, second, and fifth high intensity interval. The analysis of muscle biopsies for redox proteomics involved a differential Cys labelling step by taking advantage of a chemically equivalent heavy (d5) and light (d0) isotopic form of the common thiol alkylating reagent N-ethylmaleimide (NEM).
The aim of the study was to identify and quantify the reversible redox state of specific Cys residues within individual muscle samples and to quantify relative protein abundance between samples and to investigate both the effects of ageing and exercise. Data obtained indicate differences in protein contents between muscles of young and older subjects at rest and an acute effect of the exercise on the protein composition of muscles in older subjects only. This was associated with exercise-induced modification of the redox status of a sub-group of cysteines in the muscles of young and older subjects.

NC19
Endurance exercise and immune function: role of redox homeostasis and inflammatory biomarkers in systemic adaptation

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Regular physical activity can enhance immune function and effectively prevents the spread of the cytokine response, thus reducing systemic low-grade inflammation and improving various immune markers. Moreover, regular exercise ameliorates antioxidant defense incrementally and diminish oxidative stress in skeletal muscle and other tissues, including immune cells.
In this study we verified the efficacy of short-term endurance exercise in the adaptation of redox components in PBMCs and in the plasma cytokines. We performed a transcriptional and protein analyses of selected antioxidants (SOD1, SOD2, GPx1, TrxR1, CAT) and HSPs (HSP70, HSP27) in PBMCs from 10 physically active healthy males (26.6±3.1 years), also controlling for the level of 4-HNE and NFkB activation in PBMC, and for the protein carbonyls and cytokines' concentration (IL6, IL8, IL10, IL17E, IL17F, IL21, IL22 and IL23) in plasma. Blood samples were collected before and after (3 and 24 hrs) an acute bout of endurance exercise (70% HRmax for 30'), or a short-term endurance training (70% HRmax for 30'/day for 5 consecutive days).
A significant reduction in plasma protein carbonylation and PBMC 4-HNE was detected after 5-day training compared with the baseline levels ($p<0.05$). An early response (3hrs) in gene expression was detected for SOD1, Hsp70 and Hsp27 ($p<0.05$), followed by increase in protein levels at 24 hrs and return to basal level after 5 days, while a significant reduction in gene expression and/or protein content was identified for SOD2, GPx1 and TrxR1. This picture was paralleled by an increase in p-p65-NFκB at 24 hrs ($p<0.05$), its decrease below the baseline level at 5-day, concurrent with a decrease in the plasma level of the pro-inflammatory cytokines IL-8, IL-21 and IL-22.
This study showed that 5 days of regular exercise is effective in improving the redox homeostasis in circulating PBMCs, as well as the systemic pro-inflammatory environment in healthy adults.

NC20

Personalized multicomponent physical exercise program for the prevention and reversal of frailty in the elderly

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Objectives: The objective of this study is to analyze the effects of a personalized multicomponent physical exercise program on frailty in non-institutionalized older adults, as well as its incidence on psychological and cognitive parameters.
Methodology: 12 older adults between 72 and 84 years old followed a personalized multicomponent exercise program with elastic bands for 6 months, performing 3 sessions of 50 minutes of training per week. Each of the sessions consists of warm-up, strength (60-80% 1RM), endurance (50-75% HRmax), proprioception, and stretching. For the customization of the loads and intensities of the exercise, a comprehensive geriatric assessment is performed together with the 1RM strength tests. This evaluation is repeated once the intervention is finished.
Results: The participants significantly reduced the frailty criteria ($p = 0.0115$) and improved the Barthel scale score ($p = 0.0060$). The Tinetti fall risk scale and gait speed reflect significant improvements respectively ($p = 0.0037$ and $p = 0.0038$). Regarding body composition, the participants reduced their abdominal girth ($p = 0.0059$) and the percentage of fat mass ($p = 0.0010$). Likewise, the percentage of lean mass increased ($p = 0.0036$) and the palmar pressure force of the dominant hand ($p = 0.031$). Bradycardial adaptation to exercise resulted in a significant decrease in resting heart rate ($p = 0.0038$). At the cognitive level, the score in the Minimental test improved ($p = 0.0149$), as well as the Yesavage test for evaluating the state of depression ($p = 0.0057$).
Conclusions: The personalization of a multicomponent physical exercise program reduces the criteria of frailty present in the elderly, improves their body composition and cardiovascular health parameters. On the other hand, it prevents the risk of falls, improves the speed of walking and the emotional and cognitive state.
Keywords: Multi-component exercise, frailty, healthy aging, older adult.

NC21

Multicomponent high intensity interval training induces positive adaptations in old glucose-6-phosphate dehydrogenase overexpressing mice

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Introduction: Glucose-6-phosphate dehydrogenase (G6PD) regulates the NADPH levels, protecting the cell against oxidative damage. Mice that overexpress G6PD have higher levels of NADPH, lower levels of oxidative damage, and better protection from age-associated functional decline. High-intensity interval training is a powerful short-term stimulus to induce many of the physical adaptations typically associated with traditional, moderate-intensity long duration continuous training. Therefore, our primary purpose was to study whether multicomponent high-intensity interval training (MHIIT) would improve physical function in old mice.
Methods: Twenty-six G6PD-Tg male mice (23-months-old) were randomized into two groups: sedentary (n=9) and trained (n=17). The trained group followed a MHIIT that included motor coordination, resistance and endurance exercises. Mice were trained 5 days a week (an average of 22 minutes/day) for ten weeks. All animals were evaluated before and after the intervention for different functional parameters. Independent and paired t-tests were conducted to compare means between and within groups. Data are expressed as mean (standard error of mean). A value of $p<0.05$ was considered statistically significant.
Results: Compared to sedentary mice, trained mice showed an improvement in: (i) motor coordination (rotarod test) [114.7 (7.4) vs 163.3 (13.1) seconds, $p<0.05$]; (ii) grip strength [4.4 (0.1) vs 5.7 (0.2) grams of force / grams of body weight, $p<0.0001$]; (iii) maximal carrying load (ladder-climbing test) [95.6 (4.5) vs 206.9 (4.8) % of body weight, $p<0.0001$]; (iv) endurance (continuous-treadmill test) [1501 (265.2) vs 2679 (279.7) meters, $p<0.05$]; and (v) maximal oxygen consumption [34.85 (1.28) vs 40.11 (1.10) mL/min/$kg^{0.75}$, $p<0.05$]. Furthermore, the "Valencia Score" for frailty

showed a decrease in the percentage of frail mice in the trained group when compared to the control one (7 vs 45 %, $p<0.0001$).
Conclusion: Our results show that the novel physical training protocol we present here improves physical function and prevents, or even reverses, frailty in G6PD-Tg.

NC22
The NAD^+ precursor nicotinamide riboside improves redox homeostasis and performance in old mice

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Background: Nicotinamide adenine dinucleotide (NAD) levels decline with age, which promotes several aging-associated diseases due to its important function as a cofactor in multiple metabolic and physiological processes as well as in redox homeostasis. Dietary supplementation with NAD precursors, such as nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR) are emerging strategies that pursue to restore or maintain NAD levels conferring beneficial effects on aging and lifespan.
Objective: The purpose of this work was to study the effects of NR supplementation on functional performance, muscle metabolism and oxidative stress status in old mice.
Methods: We used a cohort of 19-month-old male C57BL/6J mice divided in two groups: NR supplemented group (n=10) received 400 mg/kg/day of NR in the drinking water for 12 weeks, and the control group (n=11). Both groups were evaluated for functional parameters (treadmill endurance test, rotarod test, grip strength test) and indirect calorimetry. Tissue samples were analysed for biochemical parameters.
Results: There were no differences in energy expenditure and respiratory quotient (indirect calorimetry parameters). Supplemented mice exhibited better endurance capacity in incremental treadmill test and motor coordination than control group, but there were no differences in grip strength test. NR supplementation achieved significant increases in NAD^+, NADH, $NADP^+$ and NADPH levels in liver tissue. Pyruvate dehydrogenase kinase (PDK4) and the phosphorylated form of PDH in Ser293 showed reduced protein levels in NR supplemented group, which would lead to an increased production of acetyl-coA and, therefore, increased energy production. Markers of lipid peroxidation (malondialdehyde, MDA) and carbonylated proteins were reduce in NR supplemented mice.
Conclusion: Nicotinamide riboside supplementation reduces oxidative stress in skeletal muscle and improves functional performance in old mice.

NC23
Apigenin and rutaecarpine target cellular senescence to prevent aging-bone phenotype in human mesenchymal skeletal stem cells

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Osteoporosis is a systemic aging-related disease that causes reduction and deterioration of bone mass, density, and microstructure leading to increased risk of bone fragility and fractures. Current pharmacological interventions to treat osteoporosis target reduction of bone resorption and induction of bone formation. However, the long-term use of anti-osteoporotic drugs exhibit side effects like increased risk factor of developing cardiovascular diseases. Thus, there is a need for new therapeutic approaches. In this study we identified Apigenin (Api) and Rutaecarpine (Rut), plant-derived antioxidants, employing a functional screen of a natural product library (144 compounds). Api and Rut were found to exert significant induction on osteogenesis of bone marrow mesenchymal stromal stem cells (BMSCs) *in vitro*. In order to determine the osteogenic effects of Api and Rut, we used an ex-vivo organotypic embryonic chick-femur culture model, on which they both significantly increased the average bone volume and cortical thickness of the chick-femur compared to control. Global gene expression profiling and protein analysis revealed the activation of a number of signaling pathways, including focal adhesion kinase (FAK), transforming growth factor b (TGFb), selenium, and oxidative stress. In addition, treatment with Api and Rut during oxidative insult with tert-butyl hydrogen peroxide, reduced the levels of the senescence-associated secretory phenotype (SASP) and senescence (P53, P16, and P21) markers. It also reduced intracellular ROS levels, measured with DCFDA, and gene expression of antioxidant defense enzymes (HMOX1 and SOD2). Furthermore, *in vitro* treatment with Api and Rut of primary hBMSCs from elderly subjects, characterized by low osteoblastic differentiation ability, significantly enhanced osteoblast formation when compared with primary hBMSCs from

young donors**.** Api and Rut enhance osteoblastic differentiation and bone formation via reducing the senescence-associated phenotype with aging and thus represent a potential therapy to reduce the progression of osteoporosis.

NC24
Physical contact between prematurely aging and non-prematurely aging mice while they cohabit is crucial to improve their oxidative state

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Aging is associated with chronic oxidative stress, which contributes to a deterioration of the homeostatic systems and a greater mortality. Nevertheless, we have recently observed that old mice improved immunity and lifespan after cohabiting with adult animals. In a model of prematurely aging mice (PAM), based on an inadequate response to stress of dealing with the novel environment of the T-maze, adult PAM show high oxidative stress and shorter longevity compared to exceptionally non-prematurely aging mice (ENPAM) of the same age. Improvements in oxidative stress in leukocytes and some organs of PAM were recently shown after living, continuously, with ENPAM for 2 months, but ENPAM showed some parameters deteriorated. The objectives of the present study were to verify if living only 15min/day for 2 months allows positive effects in PAM and also in ENPAM, as well as to find out the influence of physical contact on these effects. Female ICR-CD1 PAM and ENPAM were divided into four groups. Two control groups (PAM and ENPAM) and two experimental groups of social interaction (2 PAM and 5 ENPAM cohabiting in the same cage 15min/day for 2 months in contact or physically separated by a methacrylate wall). After this time, several parameters of oxidative stress were analyzed in peritoneal leukocytes. The results showed higher glutathione reductase activity and lower GSSG/GSH ratio and oxidative damage to lipids in leukocytes of PAM after cohabiting with ENPAM, in comparison with PAM controls. ENPAM did not show any deterioration of these parameters in comparison with ENPAM controls. However, PAM which cohabited with ENPAM physically separated, did not show these improvements in oxidative status. In conclusion, cohabiting for a short time (15 min/day for 2 months) produces significant improvements in PAM, without deteriorating ENPAM, being the physical contact essential for these positive effects of social interaction.

NC25
Akkermansia muciniphila: a new ally to combat the oxidative stress of aging

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The process of aging is characterized by a progressive and general loss of functions of the organism, resulting on the accumulation of oxidative damage. According to the oxidation-inflammation theory of aging, the redox state of immune cells is involved in the rate of aging. Currently, in the world population the number and proportion of the elderly people is increasing as well as the disabilities and diseases associated with age. This has led to an increase in the search for lifestyle strategies that slow down aging. In this context, knowing the important role of the gut microbiota in health, and consequently in the rate of aging, the use of probiotics is being considered in recent years. The "new" probiotic *Akkermansia muciniphila* can be very promising since its intestinal concentration decreases with advancing age but increases in centenarians. However, its benefit effect on immune redox state in old individuals is unknown. Thus, our aim was to investigate the effect of daily intake of *A. muciniphila* (10^8 ufc/100µL PBS), for one month, on several parameters of oxidative stress in peritoneal immune cells of ICR-CD1 old (74±4 weeks of age) mice. The results showed that old animals that ingested *A. muciniphila*, compared to those of the same age who did not take it (controls), significantly improve their antioxidant defences (glutathione peroxidase activity $p<0.05$; glutathione reductase activity $p<0.001$ and reduced glutathione concentration (GSH) $p<0.01$). These mice also showed lower values of GSSG/GSH ratio ($p<0.05$) as well as of oxidative lipid damage ($p<0.01$) than controls. In conclusion, since the redox state of peritoneal immune cells is a marker of this state in the rest of organism and of the rate of aging, the daily supplementation with *A. muciniphila* could be proposed as a useful strategy to improve aging and thus achieve healthy longevity.

NC26
Intermittent cold and hypobaric hypoxia treatment modulates oxidative stress in injured muscles of rats

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Background**:** Muscle regeneration is a coordinated complex process. The initial phase of regeneration is an inflammatory response, including redox signaling. Cold and intermittent hypobaric hypoxia had been shown to reduce oxidative stress damage in muscle and in several tissues. Based on this, the aim of this study is to evaluate redox parameters in healthy and injured contralateral gastrocnemius muscles after intermittent cold and hypobaric hypoxia treatments.
Methodology**:** After inducing injury in the right gastrocnemius of rats, animals were submitted for 4 h/day during 9 days to simultaneous hypobaric hypoxia (4500 m) and cold (4°C) (COHY). A control group (CTRL) was maintained in normoxia at 27ºC. After 9 days of treatment, gastrocnemius muscles of injured and control legs, were collected and homogenized in urea lysis buffer (6M urea, 1% SDS). 4-HNE, Nrf2, HSP70 and eNOS markers were analyzed by Western Blot.
Results**:** Our results showed a decreased level of 4-HNE after COHY treatment, for both healthy and injured legs, although no differences were found for HSP70 expression. Injured legs showed a tendency to reduce the Nrf2 expression, compromising the antioxidant response. eNOS expression increased in the injured leg under COHY treatment, which suggest a better supply of O_2 and vascularization to the tissue. Additionally, a full recovery of muscle fatigue index was found after treatment. Conclusions**:** The intermittent cold and hypobaric hypoxia treatment reduced oxidative stress damage when 4-HNE is analyzed, although further redox analysis, such as antioxidant parameters, is required. Thereby, oxidative stress modulation can play a key role in the recovery of skeletal muscle damage.
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NC27
Small extracellular vesicles from healthy cells improves cell function and stemness in premature senescent stem cells by miR-302b and HIF-1a activation

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Background: Aging is accompanied by the accumulation of senescent cells that alter intercellular communication, thereby impairing tissue homeostasis and reducing organ regenerative potential. Recently, administration of mesenchymal stem cells (MSC)-derived extracellular vesicles has proven to be more effective and less challenging than current stem cell-based therapies. Extracellular vesicles contain a cell-specific cargo of proteins, lipids and nucleic acids that are released and taken up by probably all cell types, thereby inducing functional changes via horizontal transfer of their cargo.
Methods: Here, we describe the beneficial properties of small extracellular vesicles (sEV) derived from non-senescent MSC cultured in a low physiological oxygen tension (3% O_2) microenvironment into prematurely senescent MSC cultured in a hyperoxic ambient (usual oxygen culture conditions, i.e. 21% O_2).
Results: We observed that senescent MCS treated with sEV from non-senescent MCS showed reduced SA-b-galactosidase activity levels, OSKM pluripotency factors overexpression and increased glycolysis as well as reduced OXPHOS. Moreover, these sEV cargo induced the upregulation of miR-302b and HIF-1a levels in target cells. We propose that miR-302b triggered HIF-1a upregulation, which in turn activated different pathways to delay premature senescence, improve stemness and switch energetic metabolism towards glycolysis.
Conclusions: Taken together, we suggest that sEV could be a powerful tool to restore the altered intercellular communication, improve stem cell function and stemness, thus delaying stem cell exhaustion in aging.
Keywords: Oxygen, redox, physioxia, physiological oxygen concentration, extracellular vesicles, aging, senescence.

NC28
Age- and sex-dependent changes of trace elements and redox parameters in mice

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Aging is a progressive physiological change with alterations in immune response and antioxidant capacity. Especially the essential trace elements (TE) Se, Cu, Zn, and Fe act as cofactors or prosthetic groups of antioxidant or protective enzymes, such as glutathione peroxidases or Cu/Zn superoxide dismutase. Accordingly, disturbed TE homeostasis contributes to the aging process and various diseases.
To investigate the impact of TE status, young and aged mice were analyzed concerning age- and sex-dependent differences by feeding diets with defined TE amounts. To this end, we measured the TE profiles of six TE (Se, Cu, Zn, Fe, Mn, I) in serum and liver using ICP-MS/MS along with other tissues and further functional biomarkers of the TE status.
Consumption of a TE supplemented chow diet resulted in an age-related increase of Cu and I concentrations in serum. Male mice further showed higher Zn levels in serum and increased Cu concentrations in liver whereas in females, hepatic Fe levels were increased. Considering all analyzed tissues, there was no consistent pattern of TE distributions related to age and sex. Also the Nfr2 target gene NAD(P)H:quinone oxidoreductase was differently affected in organs like heart, lung, and kidney in comparison to liver. Furthermore, thioredoxin reductase activity showed age-related changes in heart and lung, whereas activities in duodenum, liver, or kidney were not affected by age.
Murine age-dependent changes in the TE serum profile were also recognized in humans and *C. elegans* worms as well, indicating comparable age-specific TE profiles across different species. Future intervention studies will focus on the possibility to modulate the TE status of the elderly and thereby protect from age-related diseases.

NC29
Copper-mediated changes in cellular selenium metabolism

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Selenium (Se) and copper (Cu) are essential trace elements for humans with major roles in redox homeostasis by modulating enzyme activities and signaling pathways. Se is important for the expression of various selenoproteins, e.g. glutathione peroxidases (GPXs) and thioredoxin reductases (TXNRDs) which are both responsible for protection against hydroperoxides. Cu itself is a redox active element, but it is also important for superoxide dismutase 1 (SOD1) activity. While both elements are well investigated on their own, interactions have yet to be studied in detail.
We aimed to address the question how Se and Cu interfere with each other to modulate the cellular redox tone. To this end, we analysed Cu-dependent proteins, various selenoproteins, and also the cellular content of both elements. HepG2 cells were treated with 50 nM sodium selenite and with or without 100 µM copper sulfate for 72 h. After 48 h of incubation, Bathocuproine disulfonate (BCS) or Tetrathiomolybdate (TTM) were added to chelate Cu. In a mouse feeding study with different contents of Se and Cu in the diet or drinking water, liver and gastrointestinal samples were analysed.
Cellular Se content was not affected or slightly increased by combined Cu treatment. Nevertheless, Cu was able to diminish activity and expression of various selenoproteins. Moreover, parts of the selenoprotein synthesis machinery were reduced upon Cu treatment. The chelators were able to diminish cellular Cu content, but could not reverse all Cu induced effects. Besides, also Se was able to reduce mRNA level of metallothionein 2 (MT2a), which is important for cellular Cu homeostasis.
We were able to show that the effects of Cu on selenoprotein activity and expression are higher *in vitro* than in *in vivo* samples. Furthermore, Se showed interference with Cu homeostasis. The detailed mechanisms of Se and Cu interactions need to be investigated.

NC30
Distinctive under-expression profile of inflammatory and redox genes in the blood of elderly patients with cardiovascular disease

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Chronic low-grade inflammation and oxidative stress are present in most of the pathologic mechanisms underlying non-communicable diseases. Inflammation and redox biomarkers might therefore have a value in disease prognosis and therapy response. In this context, we performed a case-control study for assessing in whole blood the expression profile of a panel comprising 84 inflammation-related and 84 redox-related genes in 130 elderly subjects with various pathologies (cardiovascular disease, hypertension, dyslipidemia including hypercholesterolemia, type 2 diabetes mellitus), kept under control by polyvalent disease-specific medication. The study highlights a distinctive expression profile of genes critically involved in NF-κB-related inflammation and redox signaling in the blood of patients with cardiovascular disease, characterized by significant down-regulation of the genes *NFKB2*, *NFKBIA*, *RELA*, *RELB*, *KKT1*, *IRF1*, *STAT1*, *CD40*, *LTA*, *TRAF*2, *PTGS1*, *ALOX12*, *DUOX1*, *DUOX2*, *MPO*, *GSR*, *TXNRD2*, *H SPA1A*, *MSRA* and *PDLIM1*. This gene expression profile defines the transcriptional status of blood leukocytes in stable disease under controlled medication, without discriminating between disease- and therapy-related changes. This study brings preliminary proof on a minimally invasive strategy for monitoring disease in patients with cardiovascular pathology, from the point of view of inflammation or redox dysregulation in whole blood.
The study was funded by the European Regional Development Fund, Competitiveness Operational Program through the grant P_37_732/2016 REDBRAIN.
Keywords: aging-related diseases, cardiovascular disease, inflammation, NF-κB signaling, redox, oxidative stress.

NC31

Sulforaphane supplementation of murine insulin-resistant pregnancy normalises adaptive Nrf2 responses in offspring lung endothelial cells and protects mother and offspring from cardiometabolic syndrome

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Redox imbalance underlies pregnancies complicated by maternal obesity and insulin-resistance (IR), which prime both mother and child towards an increased risk of cardiometabolic dysfunction. In this study we investigated whether in utero potentiation of endogenous antioxidant defences, coordinated by nuclear factor E2-related factor 2 (Nrf2), can improve perinatal and offspring outcomes in a murine model of IR during pregnancy and weaning. Proven breeder C57BL/6 dams were fed an obesogenic diet (Ob) for 6 weeks before mating, and throughout pregnancy and lactation and supplemented with the dietary Nrf2 activator sulforaphane (SFN, 2.5mg/kg, daily) during pregnancy and weaning. All offspring was weaned onto normal chow. The metabolic and vascular phenotypes of dams and offspring are reported post-weaning, at 6 weeks and 6 months of age, respectively. Results are mean±SEM from 5-10 pregnancies, analysed by 2-way ANOVA or Student's *t*-test. SFN administration during pregnancy improved glucose tolerance of Ob dams post-weaning (area under the curve of glucose tolerance test: 2456±143.7 versus 3079±170, $p<0.05$) and in 6-month-old male offspring (2454±113.9 versus 1.757±144.6, $p<0.01$). Ob dam intake of SFN also reduced maximal contractile responses of isolated small mesenteric arteries to noradrenaline post-weaning (1.712±0.12 versus 2.51±0.11, $p<0.01$, mN/mm) and in 6-month-old male offspring (1.61±0.1 versus 2.30±0.11, $p<0.01$, mN/mm). Lung endothelial cells (LEC) derived from 6-week-old offspring of Ob dams supplemented with SFN, had attenuated mitochondrial reactive oxygen species (MitoSOX Red) basally and when stimulated with the lipid peroxidation product 4-hydroxynonenal (4-HNE). The Ob-SFN LEC also had normalised adaptive Nrf2 responses to 4-HNE compared to Ob group, which was assessed by protein expression of Nrf2 target antioxidant enzymes. Our findings suggest that gestational intake of dietary SFN can reverse the adverse metabolic and vascular outcomes of IR pregnancies in both dam and offspring.

NC32

Metallomic profiling in human coronary artery endothelial cells: effects of physiological oxygen levels and ischemia-reoxygenation injury

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Hypoxia and oxidative stress are contributing factors in cardiovascular disease. Zinc deficiency is a risk factor for coronary heart disease and zinc has been shown to afford protection against ischemia-reperfusion injury. Zinc and other metal homeostasis, such as iron and calcium, are intimately associated with cellular responses to changes in ambient oxygen tension and oxidative stress. We have used ICP-MS (Inductively Coupled Plasma Mass Spectrometry) to measure changes in total metal content in human coronary artery endothelial cells (HCAEC) cultured long-term under hyperoxia (18 kPa), physiological normoxia (5 kPa) and hypoxia (1 kPa O_2), and measured total zinc levels (ng/µg protein) of 0.345, 0.267 and 0.117, respectively. These findings were correlated with changes in the expression of metal transporters and binding proteins. When HCAEC were subjected to hypoxia for 1 h and reoxygenation, total zinc levels were not altered significantly. In addition, we used Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) on cultured HCAEC monolayers to monitor changes in the spatial distribution of metals and replicated our findings using ICP-MS on cell lysates. Finally, we investigated the mobilisation labile iron using the fluorescence probe FeRhoNox-1 and observed an increase in Fe^{2+} following hypoxia reoxygenation. Our study provides important insights into the critical role of metal homeostasis in vascular cell culture under defined ambient oxygen levels and therapeutic interventions that may limit damage in ischemia-reperfusion injury.
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NC33
Citrullination of histones decreases extracellular histones-mediated oxidative stress, cell death, and vascular dysfunction in endothelial cells

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Sepsis is characterized by an exacerbated response of the host against infection. One of the first events occurring in sepsis is NETosis, an immune defense mechanism characterized by the explosion of neutrophils, forming large sticky networks or "NETs", which contain DNA and nuclear proteins, mainly histones. It is known that H3 citrullination (citH3) is an essential process for the initiation of NETosis, so it is expected that large amounts of histones released are citrullinated. However, although the cytotoxic effect of extracellular histones on many cell types and tissues is partially known, the effect of citrullinated histones on endothelial cells has not been characterized yet. Our study demonstrated that citrullinated histones are less cytotoxic than their non-citrullinated counterparts. Among the mechanisms explored, we showed that citrullinated histones did not activate as much oxidative stress as native histones. Moreover, citrullinated histones caused less endothelial dysregulation and expression of immune mediators than native histones. Furthermore, we observed that citH3 levels were higher in the most severe patients (septic shock), and its levels remain high during the course of the disease, suggesting that NETosis is present during the development of sepsis.

NC34
Extracellular histones induce oxidative stress and endothelial pyroptosis leading to severe phenotypes in sepsis

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Circulating extracellular histones has gained relevance as cytotoxic mediators in the pathophysiology of sepsis, promoting cell death and tissue damage. Extracellular histones act as damage-associated molecular patterns, which induce oxidative

stress that lead to activate NLRP3 inflammasome and pyroptosis, a programmed cell death mechanism that produces inflammation. Despite inflammasome activation during sepsis in immune cells has been proposed, there is no information about the mechanism of activation of the inflammasome and pyroptosis in endothelial cells. Our study highlights the role of extracellular histones inducing NLRP3 inflammasome activation and pyroptosis in endothelial cells. Likewise, we demonstrated how histone hyperacetylation attenuates this process. Furthermore, we demonstrated that pyroptosis occurred in septic shock patients and circulating histone levels correlate with the expression of pro-inflammatory cytokines, the release of endothelial adhesion factors and septic shock severity. We propose histone-mediated pyroptosis as a new target to develop therapeutic interventions.

NC35
Obesity causes PGC-1α deficiency in exocrine pancreas leading to IL-6 upregulation via NF-κB in acute pancreatitis

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Obesity increases the risk of local and systemic complications during acute pancreatitis. Our aim was to study PGC-1α levels in pancreas from lean or obese mice and to determine the role of transcriptional coactivator PPARγ coactivator 1α (PGC-1α) in the inflammatory response during acute pancreatitis. Lean and obese C57BL6/J mice were studied first; subsequently, wild-type and PGC-1α knockout (KO) mice with cerulein-induced pancreatitis were used to assess the role of PGC-1α in the inflammatory response during acute pancreatitis. PGC-1α protein levels were markedly diminished in pancreas of obese mice. PGC-1α protein levels increased in pancreas of lean mice with acute pancreatitis, but not in obese mice with pancreatitis. In lean mice with pancreatitis increased PGC-1α protein levels were acetylated and the mRNA expression of its target genes superoxide dismutase-2, peroxiredoxin 3 and catalase were downregulated. PGC-1α deficiency markedly enhanced nuclear translocation of phospho-p65 and recruitment of p65 to interleukin-6 (*Il-6*) promoter leading to increased *Il-6* mRNA levels in these mice. In wild-type mice PGC-1α bound phospho-p65 in pancreas during pancreatitis. Edema and the inflammatory infiltrate were more intense in the pancreas from PGC-1α KO after cerulein-induced acute pancreatitis in comparison with wild-type mice. In addition, PGC-1α KO mice exhibited increased IL-6 plasma levels than wild-type mice with pancreatitis. In conclusion, obese mice exhibit PGC-1α deficiency in the pancreas. PGC-1α acts as selective repressor of nuclear factor-κB (NF-κB) towards IL-6 during acute pancreatitis. PGC-1α deficiency causes a severe inflammatory response during acute pancreatitis through NF-κB-dependent upregulation of *Il-6*.

NC36
Nitration of cystathionine β-synthase in acute pancreatitis

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Acute pancreatitis (AP) is an acute inflammatory disorder of the pancreatic gland that often leads to local and systemic complications. AP is one of the main causes of hospital admission for gastrointestinal disease in Western countries, and its incidence has increased dramatically over the past decade. Several evidences suggest that dysregulation of the trans-sulphuration pathway may contribute to the pathogenesis of AP. Based on these backgrounds, the aim of this work was to study the methionine cycle as well as the trans-sulphuration pathway using a metabolomic and proteomic approach in an experimental model of cerulein-induced acute pancreatitis in mice. AP triggered marked depletion of methionine, S-adenosylmethionine, 5'-methylthioadenosine, cystathionine, cysteine, and reduced glutathione (GSH) in pancreas, without changes in S-adenosylhomocysteine and homocysteine levels. Proteomic analysis of the enzymes involved in the trans-sulphuration pathway showed increased levels of adenosylhomocysteinase and cystathionine gamma-lyase but S-methyl-5'-thioadenosine phosphorylase levels diminished in pancreas with AP. Although cystathionine-β-synthase (CBS)

protein levels did not change with acute pancreatitis, *Nos2* mRNA expression was up-regulated and caused tyrosine nitration of CBS in pancreas. In conclusion, tyrosine-nitration of cystathionine β-synthase blockades the trans-sulphuration pathway in acute pancreatitis promoting GSH depletion.

NC37
NAD^+ boosters reduce the oxidative, apoptotic and inflammatory status of leukocytes from rheumatoid arthritis patients

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NAD^+ is an important cofactor/second messenger for key cellular processes whose modulation might have a therapeutic role in Rheumatoid Arthritis (RA).
Aims: 1- To study the NAD^+ metabolism in RA patients. 2- To analyze the effect of NAD^+ boosters in leukocytes from active RA patients.
Plasma and PBMCs were purified from 100 RA patients and 50 healthy donors (HDs). NAD^+ levels were determined by using the NAD/NADH-Glo Assay. NAD^+-consuming genes expression were analyzed by RT-PCR. In parallel, PBMCs from six HDs and six active RA patients were treated *ex vivo* with 1 mM of NAD^+ boosters including nicotinamide (NAM), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN). After 24 hours, intracellular reactive oxygen species (ROS) levels (DFCHDA) and the percentage of apoptotic PBMCs (annnexin V/PI) were assessed by flow cytometry. Finally, a panel of pro-inflammatory genes were evaluated by RT-PCR.
NAD^+ levels were significantly reduced in plasma of RA patients compared with HDs and directly related to disease activity. Accordingly, the expression levels of genes involved in the consumption of NAD^+ such as SIRT-1, CD38 and PARP-1 were found up-regulated in RA PBMCs. PBMCs isolated from RA patients showed an increased oxidative, apoptotic and proinflammatory status compared with HDs. The in vitro treatments with NAD^+ boosters significantly increased the NAD^+ levels and promoted a deep reduction of intracellular ROS, the percentage of apoptotic cells and the expression levels of key inflammatory mediators, such as IL-6, IL-8, IL-1b, TNF-α, CCL2, IL-23, and STAT-3.
Conclusions: 1. NAD^+ metabolism is altered in RA, involving both, reduced NAD^+ levels and increased expression of NAD^+-consuming genes. 2. NAD^+ boosters reduced the oxidative, apoptotic and inflammatory profile of RA leukocytes through the parallel increase of intracellular NAD^+ levels. Thus, NAD^+ boosters might be considered novel therapeutic tools for RA patients.
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NC38
Phenotypic changes to vascular smooth muscle cells induced by oxidant-modified extracellular matrix

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Inflammatory cells produce oxidants at sites of inflammation to kill invading pathogens. Since these oxidants are not specific, they also cause collateral damage to host tissues. In atherosclerosis, a chronic inflammatory disease, the developing plaque contains high numbers of activated inflammatory cells, and oxidized lipids and proteins. Extensive oxidation and nitration have been detected on extracellular matrix (ECM) proteins of the artery wall in all stages of atherosclerosis. The vascular ECM is important for the integrity and function of the artery wall, and also determines vascular cell phenotype. During atherogenesis, vascular smooth muscle cells (VSMCs) undergo a pronounced phenotypic switch from quiescent and contractile, to a proliferative and synthetic form. We hypothesized that ECM modification by inflammatory oxidants drives this phenotypic switch, and thereby contributes to atherosclerosis development.
ECM from cultures of primary human coronary artery smooth muscle cells (HCASMCs) was treated with increasing concentrations of the inflammatory oxidant, peroxynitrous acid (ONOOH), and the ONOOH-producing compound SIN-1, with ELISA used to determine ECM modifications. Cell adhesion to native and modified ECM, and subsequent proliferation, were examined using calcein-AM staining and MTS assay. Expression of mitosis, ECM and inflammatory genes was examined by qPCR.

Extensive modification and nitration of ECM components was detected on HCASMC-ECM treated with ONOOH. Nitration was also detected with SIN-1 though at lower levels. ONOOH-modified ECM reduced cell adhesion, but increased proliferation of HCASMCs. Gene expression of ECM proteins (laminin, fibronectin, and versican), inflammatory cytokines (IL-1β and IL-6), and vascular cell adhesion molecule (VCAM-1) were up-regulated, consistent with ECM remodeling and a pro-inflammatory state.
This study suggests a mechanism through which inflammation-induced ECM-modification may contribute to phenotypic switching of VSMCs, a key step in the formation of atherosclerotic plaques, and highlights the targeting of oxidant formation as a potential preventative, or therapeutic, strategy for atherosclerosis.

NC39
Adduction reactions of dimethylfumarate: a 60-year-old drug with potent but poorly understood mechanisms of action

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Dimethyl fumarate (DMFU) was introduced in 1959 as a treatment for psoriasis, and subsequently in 2013 for multiple sclerosis (MS), as psoriasis patients that suffered also from MS, noticed a beneficial effect of DMFU for both diseases. The anti-inflammatory and protective properties of DMFU may also be of benefit in other diseases. However, such repurposing is confounded by its incompletely understood molecular mechanisms of action. Untangling the molecular actions of DMFU is a challenge caused by its broad reactivity towards biological targets which include protein cysteine residues. We hypothesized that investigation and determination of the rate constants and selectivity of DMFU reactions may shed light on its biological effects. Previous studies have reported that DMFU targets multiple species including GSH, GAPDH and Keap1 and that these processes involve Michael addition reactions. We have determined kinetic data for reaction of DMFU with these species, with the values ranging from 0.5 - 0.8 M^{-1} s^{-1} for low molecular mass thiols (*N*-Ac-Cys and GSH, respectively), 0.4 M^{-1} s^{-1} for BSA, 2.2 M^{-1} s^{-1} for GAPDH, to 23 M^{-1} s^{-1} for Cys residues on (wild type) Keap-1. Mutation of specific Cys residues on Keap-1 decreases the rate constant significantly indicating a strong selectivity for specific Cys residues on this protein. Unlike other alpha,beta-unsaturated carbonyls, the rate constants for adduction of DMFU to Cys residues do not correlate with the pKa of the Cys residue, indicating that the other factors such as steric and / or electronic interactions, rather than the ionization state of the thiol (i.e. the RS^- : RSH ratio), play a critical role in determining the reaction kinetics. These data indicate that some proteins, and protein Cys residues, react particularly readily with DMFU, and this may help rationalize the use of this compound as a therapeutic agent.
Keywords: Dimethyl fumarate, Keap1, Second order reaction rate constant, alpha beta unsaturated aldehydes

NC40
Vascular barrier protective effects of antioxidant white-wine pomace products against *Listeria monocytogenes* in endothelial cells

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Listeria monocytogenes is a food-borne pathogen that causes listeriosis, a disease with low incidence but a high case-fatality rate. It has been shown that *L. monocytogenes* induces endothelial barrier dysfunction via activation of pro-inflammatory cytokines, resulting from the redistribution of junctional proteins via activation of Nrf2/ARE. In previous studies, we observed that the bioavailable wine pomace products (WPP) extract, with high antioxidant properties, were able to prevent the dysregulation of membrane permeability and vascular remodelling of endothelial cells due to their antioxidant capacity and modulation of oxidative stress by Nrf2/ARE. This study investigated the protective effects of white wine pomace products in Caco-2 against infection and mislocalization of junctional proteins by *Listeria monocytogenes*. The pre-treatment of the epithelial cells with WPP bioaccessibility fractions (gastrointestinal and colonic fermented), showed an antivirulence effect, reducing the invasion percentage. Furthermore, the study of junctional proteins showed an increased expression of ZO-1 and E-cadherin in the cells treated with the WPP. These results indicate that WPP prevents *L. monocytogenes* infection, avoiding the translocation of junction protein involved in the intestinal epithelial barrier. The authors thank the financial support of Ministry of Science, Innovation and Universities Spanish State Research Agency and European Regional Development Fund (Project PGC2018-097113-B-I00).

NC41

Acute dietary nitrate supplementation modulates the inflammatory response induced by endotoxin and phorbol-miristate

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Dietary nitrate supplementation is an emerging treatment strategy to alleviate the symptoms of metabolic syndrome affections and to improve vascular function. In this double-blind crossover trial, metabolic syndrome patients performed two exercise tests for 30 min at 60–70% maximal heart rate after the intake of a nitrate-rich beverage or a placebo beverage 30 min before exercise. Blood samples were taken previously and 30mintes after the exercise test; plasma was obtained and it used to analyze nitrate-plus-nitrite levels; peripheral mononuclear cells (PBMCs) were isolated and used to analyze their inflammatory response to lipopolysaccharide endotoxin (LPS) and also to Phorbol 12-myristate 13-acetate (PMA). PBMCs were cultured for 2h at 37ºC in a medium containing or not containing LPS or PMA. At the end, the mARN was extracted, transcribed and used to analyze the expression of glutathione peroxidase (GPer), catalase (Cat), interleukin 6 (IL6) and tumor necrosis factor alpha (TNF□).
Post-exercise nitrate-plus-nitrite levels increased significantly over pre-exercise values after ingesting the nitrate-rich beverage, but remained at the pre-exercise level after ingesting the placebo beverage. Dietary nitrate increased the bioavailability for nitric oxide biosynthesis in plasma.
LPS enhances the IL6 expression in PBMCs both previous and after acute exercise performed by subjects with metabolic syndrome, but no influence on the expression of TNFα, Cat and GPer was observed. Dietary nitrate intake, previous to acute exercise enhances the expression of GPer in PBMCs after their stimulation with PMA; in the opposite, dietary nitrate intake avoids to enhancing expression of TNFα in PBMCs stimulated with PMA.
LPS induce a mild Inflammatory response to 'ex vivo' PBMCs from metabolic syndrome patients. The dietary supplementation with inorganic nitrate enhances the expression of antioxidant enzyme defenses and it reduces the inflammatory response of 'ex vivo' PBMCs induced by PMA from metabolic syndrome patients.

NC42

An increase in mitochondrial DNA copy number was observed in monocyte cell line differentiated into macrophages but not in mitochondrial respiratory protein mRNA levels and TFAM

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Background: Mitocondorial DNA copy number (mtDNAcn) levels have been reported to be negatively correlated with aging and various diseases such as Parkinson's disease. Exercise was reported to increase mtDNAcn both in muscle and in the circulation. Although changes in mtDNAcn have been reported in various conditions, whether or not the level of mtDNAcn affect the levels of mRNA and/or proteins coded in mtDNA remains under debate. As a first step toward understanding the integrated processes to control mtDNAcn and associated downstream phenomena in monocytes, we studied the PMA-induced differentiation of THP-1 cells. We analyze mtDNAcn and various transcription factors that control mtDNAcn, as well as mRNAs coded in mtDNA and CoQ10.
Method: We used human monocytic leukemia cell line THP-1. This cell line can be induced to macrophage by treatment with phorbol-12-myristate-13-acetate (PMA).
The differentiation of PMA treated cells was enhanced after the initial 3-day stimulus with PMA by removing the PMA-containing media and then incubating the cells in fresh RPMI 1640 (10% FBS) for a further 5 days.
Result and discussion: PMA administration increased mtDNAcn. Nevertheless, levels of mRNAs encoded in mtDNA were rather decreased. Levels of mitochondrial transcription factor A (TFAM) also decreased. Levels of coenzyme Q10 stayed unchanged. These results imply that, although mtDNAcn is considered as a health marker, level of mtDNAcn is not always parallels parameters of mitochondrial biogenesis.
Keywords: Mitochondrial DNA, CoenzymeQ10, TFAM, Macrophage

NC43

Quercetin protects SH-SY5Y cells against sterigmatocystin-induced toxicity: prevention of NF-κB nuclear translocation and down regulation of HO-1 expression

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Sterigmatocystin (STE) is a mycotoxin commonly detected in food and feed commodities. It has been shown that STE is able to induce different toxicological effects. Among these, the induction of oxidative stress has been demonstrated to play an important role. However, the sequence of molecular events following STE exposure have not been well characterized in literature. Furthermore, limited studies have been reported about the efficacy of antioxidants to prevent the toxic effects induced by STE exposure. The aim of the present study was to understand the sequence of some molecular mechanisms for STE-induced toxicity and the cytoprotective effect of quercetin on human neuroblastoma SH-SY5Y cells. Pretreatment of cells with 10 μM quercetin resulted in abolishing the decrease in cell viability induced by 24 h of exposure to STE (from 0.19 to 25 μM), as demonstrated by MTT assay. Concerning the molecular mechanism underlying STE toxicity, results showed that in cells exposed for 24 h to STE (0.78, 1.56 and 3.12 μM) a concentration-dependent translocation of NF-κB into the nucleus was induced, as measured by immunofluorescence. The transcription factor NF-κB is involved in the regulation of the cellular stress response, through the modulation of the expression of different sets of targets genes, such as HO-1. Thus, the effect of STE exposure on HO-1 gene expression was measured by qPCR technique. The STE induced a significant upregulation of HO-1 gene expression levels in a concentration-dependent manner. On the other hand, pre-treatment with quercetin reduced NF-κB migration into the nucleus, as well as downregulated the expression of HO-1. In summary, the results of the present study demonstrate for the first time that quercetin exerts cytoprotective effects on STE-induced toxicity in SH-SY5Y cells.
Keywords: Sterigmatocystin; Quercetin; Antioxidants; NF-κB; HO-1.

NC44
Antimicrobial and anti-inflammatory effects of volatile oils widely used in Mediterranean diet

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Background: Fennel, cumin, marjoram, anise, caraway and lavender are important nutraceuticals in the Mediterranean diet. Their volatile oils have beneficial effects on the skin, digestive system, lungs, liver, metabolism, and nervous system. However, there is a lack of knowledge on volatile oils antimicrobial, and immunologic effects on neutrophils function.
Methods**:** Volatile oils were obtained from fennel (Foeniculum vulgare) and cumin (Cuminum cyminum), marjoram (Origanum majorana), lavender (Lavandula officinalis), caraway (Carum carvi), and anise (Pimpinella anisum) by hydrodistallation. Antimicrobial activity was evaluated using the dilution method against Staphylococcus aureus, Escherichia coli, Candida albicans, Vibrio harveyi, Pseudomonas aeruginosa, and Bacillus subtilis. The anti-inflammatory model utilized respiratory burst, degranulation and calcium assays in human neutrophils using various inducers. The antioxidant activity of the essential oils was measured using xanthine oxide, ABTS, and DPPH assays. The composition of volatile oils was assessed by GC-MS analysis compared with hydrocarbon standard using the Kovats retention index.
Results: Cumin demonstrated moderate antimicrobial effects against *E. coli* (-), *V. harveyi* (-) and *B. subtilis* (+) while fennel, marjoram, and lavender showed effects against *S. aureus* and *E. coli* at higher concentrations. Cumin and fennel volatile oils inhibited fMLF/CB- and MMK-1-induced superoxide generation and elastase release in human neutrophils. Cytotoxicity and free-radical scavenging effects were not responsible for the observed effects. Moreover, the downstream pathway of human neutrophils activation was also suppressed by both volatile oils including calcium and MAPK expression. The major volatile components of fennel and cumin were anethole (over 70%) and cuminaldehyde (over 50%), respectively.
Conclusion: Cumin exerted antimicrobial effects while both cumin and fennel volatile oils inhibited respiratory burst and degranulation in FPR1/2 agonist-induced human neutrophils. Cumin and fennel thus might have therapeutic potential for the treatment of neutrophilic inflammatory diseases such as COPD, psoriasis.
Keywords: volatile oil; fennel; cumin; FPR receptor, anti-inflammatory; antimicrobial

NC45
Metabolic remodeling results in alterations in the redox responses of human dermal fibroblasts

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Background: Alterations in redox homeostasis are associated with the progression of aging and different human pathologies. Antioxidants (AO) may be beneficial but clinical trials often failed despite promising *in vitro* assays, suggesting that preclinical assays should be better designed to anticipate *in vivo* results. As common cell culture assays generate artificial redox environments, and mitochondria are critical players on redox homeostasis, we aim to evaluate redox responses under different metabolic contexts, by modulating the reliance on mitochondrial energy production in vitro.
Methods**:** Normal Human Dermal Fibroblasts (NHDF) were cultured in the absence of glucose (stimulating oxidative phosphorylation, OXPHOS) and compared with cells cultured in high glucose (HG) or low glucose (LG) medium, to determine how different metabolic contexts can influence the cellular effects of oxidants, specifically H_2O_2 and *tert*-butyl hydroperoxide (*t*-BHP). Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were assessed using the Seahorse XFe96, mitochondrial network morphology was evaluated by confocal microscopy, and substrate oxidation was determined using Biolog Metabolic Phenotype Microarrays. Oxidative stress was evaluated by using fluorescent dyes.
Results: Up-regulation of OXPHOS resulted in increased sensitivity to classical mitochondrial toxicants, mitochondrial mass, respiration, and oxidation of Krebs cycle substrates. Adaptation to increased OXPHOS-reliance resulted in higher basal oxidative stress, while showing increased resistance to oxidants.
Conclusion: Manipulation of substrates in the cell culture medium remodels responses to oxidative stress, which is relevant in the context of pre-clinical studies involving redox agents.
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NC46
Microgravity induces cellular senescence in human keratinocytes

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Altered gravity would be expected to have profound impacts on different human organs and tissues, including the cardiovascular system, the skeletal tissue, the central nervous system, or muscle tissues in terms of an accelerated senescence process. Although it has been demonstrated that also the skin can be susceptible to a prolonged altered gravitational force, the knowledge about the effects of microgravity on the cutaneous tissue has been little investigated.
To directly study the effects of microgravity on human keratinocytes senescence, the cells were placed for 6, 24, and 48 hours on Random Positioning Machine (RPM) device which is able to simulate a decrease of earth gravity, and cellular senescence-related markers were analyzed. Results showed that microgravity was able to reduce cell proliferation and growth capacity. Cellular morphology analysis by Transmission Electron microscopy (TEM) demonstrated alteration in keratinocytes from 6 to 48 hours. In addition, microgravity exposure increased the expression of the senescence-associated protein (i.e. p21 and p16) and an increased expression of specific senescence cytokines (i.e. IL1α, IL 6, and IL8), suggesting the induction of the senescence-associated secretory phenotype (SASP). Finally, the electrophysiological approach shows that microgravity decreases the total membrane current of human keratinocytes, indicating a more generic effect on the cellular homeostasis system.
In conclusion, the current work brings new insights on the effects of altered gravity on human skin; in particular, our results demonstrated that microgravity deeply alters the characteristic of human skin cells promoting ASSP phenotype. Further analysis of the molecular mechanisms by which microgravity induces cellular senescence, will be investigated to appreciate the impact of the gravitational forces on human skin aging and for developing effective countermeasures.

NC47
Antimicrobial peptides as possible new players in pollution-mediated skin damage

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Ozone (O_3) exposure has been reported to contribute to various cutaneous inflammatory conditions, such as eczema, psoriasis, rush etc. via a redox-inflammatory pathway. O_3 is too reactive to penetrate cutaneous tissue; it interacts with lipids present in the outermost layer of skin, resulting in formation of oxidized molecules and hydrogen peroxide (H_2O_2). Interestingly, several inflammatory skin pathologies demonstrate altered levels of antimicrobial peptides (AMPs). These small, cationic peptides are found in various cells, including keratinocytes, eccrine gland cells, and sebocytes. Classically, AMPs function as antimicrobial agents. Recent studies indicate that AMPs also play roles in inflammation, angiogenesis, and wound healing. Since altered levels of AMPs have been detected in pollution-associated skin pathologies, we hypothesized that exposure to O_3 could affect the levels of AMPs in the skin. We examined levels of AMPs using qRT-PCR, Western blotting, and immunofluorescence *in vitro* (human keratinocytes), *ex vivo* (human skin explants), and *in vivo* (human volunteer subjects exposed to O_3) and observed increased levels of all the measured AMPs upon O_3 exposure. In addition, *in vitro* studies have confirmed redox regulation of AMPs in keratinocytes. This novel finding suggests that targeting AMPs could be a possible defensive strategy to combat pollution-associated skin pathologies.

NC48

Reductive stress in the endoplasmic reticulum caused by Ero1α S-nitrosation accelerates cell senescence

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Redox homeostasis in cells is crucial for the function of biomacromolecules. Oxidative stress is known to mediate many cellular signal transduction in physiological and pathological processes, however, the biological effects and mechanisms of reductive stress (RS), an abnormal increase in electron pressure or reducing equivalents (GSH/GSSG; NADH/NAD^+; NADPH/$NADP^+$) are still poorly understood, and the role of ER RS in aging remains unknown.

We focused on our research on the endoplasmic reticulum (ER), which is sensitive to RS, and established an ER-specific RS cell model. We detected ER redox change during the aging process using ER-specific genetically encoded redox fluorescent probes, and explored the effect and mechanism of ER reductive stress on cell senescence. Quantitative S-nitrosation proteomic analysis was employed to identify the S-nitrosated cysteine residues of Ero1α with combination of IBP and LC-MS/MS analysis. we aslo determine Ero1α activity by monitoring the oxygen consumption rate via an oxygen electrode. Further study of the effect of ER-specific supplemental oxidizing power on aging was investigated.

ER exhibited reductive stress in senescent cells, and reductive stress accelerated senescence. Further study showed that the decrease of Ero1α activity is an important cause of ER reductive stress in the process of aging. For mechanism study, we found the Ero1α S-nitrosation increased in senescence cells, and Ero1α C166 and C131 were identified as the site of S-nitrosation and resulted in decreasing of Ero1α activity. ER RS results in proteostasis disruption and ER UPR compromise and lead to aging. Overexpression of Ero1αC104/131A (active form) in the initial stage of ER reductive stress significantly increased the GSSG/GSH ratio in the ER as detected by sf-roGFP$_{ER}$ probe, leading to reversal of reductive stress and delaying aging.

ER reductive stress was caused by Ero1α S-nitrosation in aging, and ER reductive stress led to protein homeostatic imbalance and decreased ER folding ability which accelerated aging, and delaying aging was successfully achieved by increasing the oxidative power in the ER. Our data demonstrate a new mechanism that Ero1α *S*-nitrosation caused ER RS and promoted cellular senescence, providing proof-of-concept that maintaining ER oxidizing power could be the future anti-aging strategy.

NC49
NALP1 involvement in cigarette smoke induces cutaneous inflammation

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Cigarette smoke alters biological processes in the skin such as redox homeostasis and inflammation response that might be involved in promoting skin inflammatory diseases. Exposure to cigarette smoke has also been linked to a destabilization of lung epithelium inflammasome, resulting in a more vulnerable immunological response to several exogenous and endogenous stimuli, including ROS. Thus, cigarette smoke has an adverse effect on host defense, increasing the susceptibility to develop infections and pathologies. In the skin, inflammasome disorders have been linked to an increasing number of diseases such as melanoma, psoriasis, vitiligo, atopic dermatitis and acne. The inflammasome protein NALP1 is an important innate immune sensor in human keratinocytes, and, together with ASC and caspase-1, it mediates the

activation and secretion of the proinflammatory cytokines IL-1b and IL-18. However, the role of CS in the NALP1 inflammasome in the cutaneous barrier has still not been investigated yet. In the present work we aim to understand how cigarette smoke exposure is involved in cutaneous NALP1 inflammasome by the use of both, 2D and 3D skin models. Results showed that CS was able to induce an oxidative damage measured by 4-HNE protein adducts, ROS and protein carbonyls. In addition, increase in pro-inflammatory markers was also determined in CS exposed skin models. To understand whether CS induced cutaneous inflammation proceeds via an NALP1-dependent or independent mechanism, the mRNA levels of NALP1, ASC, CASP1, pro-IL-1β, IL-18 as well as the protein levels of Caspase-1, ASC and NALP1 and IL-1β and IL-18 in the culture media were analyzed. Our results were able to confirm the involvement of NLRP1 in CS induced skin inflammatory response suggesting new possible therapeutic interventions aimed to prevent NLRP1 activation.

NC50
Ascorbic acid and rutin cooperation in protecting of the proteome of UV irradiated fibroblasts cultured in a three-dimensional system

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Ascorbic acid and rutin combination that is known from the oral preparations, due to their antioxidant and anti-inflammatory properties, can be used to protect skin cells against the effects of UV radiation from sunlight. Therefore, the aim of this study was to examine the cooperation of ascorbic acid and rutin in the protection of proteomic profile in UVA [dose 20 J/cm^2] and UVB [200 mJ/cm^2] irradiated human skin fibroblasts grown in a three-dimensional (3D) system.
The proteomic data obtained using SDS-Page/nanoHPLC/QExactiveOrbiTrap showed that cooperation of ascorbic acid and rutin lead UV-irradiated fibroblasts to overexpression of proteins involved in the biosynthesis and maturing of new proteins, including DNA organization, splicing and folding processes. This cells reaction was not observed in the case of cells treated with these compounds separately following UV irradiation. Moreover, the synergistic antioxidant effect of ascorbic acid and rutin even two times stronger than used separately prevented the protein modifications by lipid peroxidation products, observed mainly as decreased 4-oxynonenal-proteins adducts formation, as well as protection enzymatic proteins against such modifications moving them to the binding proteins. However, ascorbic acid stimulated rutin-protein adducts supports intracellular signalization and antioxidant system based on Nrf2 activity, what additionally protected skin fibroblasts against UV-induced oxidative stress.
Obtained results showing synergistic effect of ascorbic acid and rutin based not only on their antioxidant activity but also their impact on proteomic profile proved that this mixture creates a potentially effective protective system against skin damages caused by UV radiation.
This study was financed by the National Science Centre Poland (NCN) grant no. 2017/25/N/NZ7/00863. Cooperation between coauthors is financed by the Polish National Agency for Academic Exchange (NAWA) as part of the International Academic Partnerships (PPI/APM/2018/00015/U/001).

NC51
Cannabidiol effect on the proteomic profile of keratinocytes isolated from rat skin exposed to UVA and UVB radiation

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UV radiation, which is one of the most harmful physical factors affecting the redox status of epidermal cells, is increasingly used in phototherapy for skin diseases, including psoriasis. Therefore, the aim of this study was to analyze the effect of phytocannabinoid - cannabidiol (CBD) on the proteomic profile of keratinocytes of nude rats (RH-FOXN1^{RNU}) after skin exposure to UVA/B radiation. Animals were exposed to UV radiation for 4 weeks every 2 days in increasing doses from 0.5 to 5 J/cm^2 (UVA) or from 0.02 to 2 J/cm^2 (UVB), and every 12 hours topically treated with CBD. The proteomic profiles of isolated from epidermis keratinocytes were analyzed using SDS-Page/nanoHPLC/QExactiveOrbiTrap.
The obtained proteomic data showed that CBD, to a different extent, depending on the type of irradiation, prevents the changes caused by the UV-induced proteomic profile in keratinocytes. CBD especially restores the level of transporting proteins, mainly importins, whose expression is inhibited after UV radiation. Moreover, CBD partly prevents the reduction of protein expression through the activity of the enzyme regulator, however, its activity significantly reduces UV-induced expression of inflammatory proteins (including NFκB), but also in intracellular signal transduction (such as kinases) and DNA repair/transcription (e.g., 5',3'-nucleotidase, DNA 9 helicase binding protein), which may suggest a reduction in the harmful UV effects caused by CBD.

Obtained results showing CBD effect on skin keratinocytes proteome might be the basis for creating combined therapies for treatment skin disease associated with disorders in expression of proteins involved mainly in inflammation, intracellular signaling and DNA binding.
This study was financed by the National Science Centre Poland (NCN) grant no.2016/23/B/NZ7/02350. Cooperation between coauthors is financed by Polish National Agency for Academic Exchange (NAWA) (PPI/APM/2018/00015/U/001). SA is supported by the project European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No754432.

NC52
Effect of cannabidiol on phospholipid metabolism in keratinocytes from patients with psoriasis

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Psoriasis is a chronic inflammatory skin disease whose pathogenesis is associated with oxidative stress. Therefore, the purpose of this study was to assess the effect of cannabidiol (CBD), a phytocannabinoid with antioxidant and anti-inflammatory properties, on changes in redox balance and phospholipid metabolism in keratinocytes, isolated from the skin of patients with psoriasis and healthy people, and UV-irradiated. CBD, as a lipophilic compound, accumulates mainly in the membranes of keratinocytes, especially those from patients. This phytocannabinoid reduces the production of superoxide anion in keratinocytes from healthy people especially exposed to UVA/UVB, while in keratinocytes from patients CBD reduces generation of this radical only after UVA irradiation. Cannabidiol reduces the effectiveness of the GSH-dependent antioxidant system, while enhances the Trx-dependent system in keratinocytes of healthy individuals, while the reverse trend in patient's keratinocytes. Moreover, CBD increases the activity of catalase in keratinocytes of patients. As a consequence, in keratinocytes from patients with psoriasis, CBD significantly reduces the level of lipid peroxidation products (8-isoprostanes and 4-HNE) as well as 4-HNE-protein adducts, particularly in patients' keratinocytes irradiated with UVA. It also affects the level and metabolic activity of endocannabinoids with a tendency to reduce AEA and PEA levels in keratinocytes of healthy people and increase AEA and lower PEA levels in patients' keratinocytes. CBD modifies the metabolic activity of endocannabinoids, primarily associated with the activation of CB1/2 receptors, mainly increasing CB1 receptor expression and reducing CB2 receptor expression in patient keratinocytes, also after UV irradiation. Because CBD tends to prevent UV-induced metabolic changes in keratinocytes in healthy people, it can support the treatment of psoriasis patients with UV radiation.
This study was financed by the National Science Centre Poland (2016/23/B/NZ7/02350). Cooperation between coauthors is financed by the Polish National Agency for Academic Exchange (NAWA) (PPI/APM/2018/00015/U/001).

NC53
Active compounds combination ameliorates CFA-induced inflammatory model by attenuating macrophage-mediated localized response and nitrative damage

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Background: To investigate whether the combination of the active compounds from *Rehmannia glutinosa* (PA) switches local immunological response in CFA-induced plantalgia models to alleviate localized inflammatory response and nitrative damage.
Methods: Sixteen male C57BL/6N mice (~8 weeks) were randomly and equally divided into were induced by CFA in model and treatment groups. The plantalgia model was induced by injection of complete Freund's adjuvant (CFA) in the right hind paw. The treatment group was orally administrated with PA from day 0 to day 7 (30 minutes post-CFA on the first day). Mechanical allodynia and Thermal hyperalgesia were measured. The immunohistochemical (IHC) method was applied to quantify the M1 and M2 macrophage phenotype biomarker and M2/M1 ratio in local subcutaneous tissue.
Results: The results indicated compared with the model group, the Treatment groups Ameliorates the inflammatory pain was increased obviously. The IHC staining quantifies results indicated compared with sham EA group, The M1/M2 ratio and M1 phenotype in the EA group was significantly reduced ($P<0.05$). The inflammatory-related cytokines such as IL-2, IL-6, TNF-α were reduced after PA treatment.
Conclusion: Nitrative damage and PA could result in improving analgesic effects. Nitrative damage and PA had a positive impact on the CFA-induced model, which may increase the polarization of M2 macrophages. And enhanced the localized

expression of M2 related markers and might have an advantage on switching the M1/ M2 ratio to tissue protection and pain release and nitrative damage.
Keywords: CFA-induced model, nitrative damage, macrophage polarisation, pain management

NC54
Anti-inflammatory and anti-oxidant effects of ranolazine in astrocytes in primary culture

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In medical practice, ranolazine is used as an antianginal and antiarrhythmic drug. Favourable effects on the nervous system have also been described as antiepileptic and in the recovery of cognitive processes and it has antidiabetic and anti-inflammatory effects. Based on these effects, we have studied the response to ranolazine of astrocytes and neurons in primary culture.
Different concentrations of ranolazine were used (10^{-7}, 10^{-6} and 10^{-5} M) and added to rat neurons and astrocytes in primary culture for 24 hours. We measured the inflammatory mediators IL-β and TNF-α using ELISA technique. In addition, the protein expression levels of PPAR-γ, Mn-SOD and Cu/Zn-SOD were determined using the Western blot technique. Under these experimental conditions, ranolazine produced a decrease in pro-inflammatory mediators (IL-1β and TNFα) in astrocytes in primary culture but not in neurons. Furthermore, ranolazine, only in astrocytes, increased the expression of the anti-inflammatory protein PPAR-γ. Looking for changes in antioxidant proteins, we detected a significant increase in Cu/Zn-SOD and Mn-SOD proteins after the addition of ranolazine (10^{-7}, 10^{-6} and 10^{-5} M) in astrocytes in primary culture.
In conclusion, ranolazine, at concentrations corresponding to those used in clinical practice, inhibited the genesis of pro-inflammatory mediators such as IL-β and TNF-α, promoted the expression of anti-inflammatory proteins such as PPAR-γ and increased the antioxidant proteins Mn-SOD and Cu/Zn-SOD. These effects were evidenced only in astrocytes in primary culture.

NC55
Effects of aspirin on inflammation and oxidative stress induced by $A\beta_{1-42}$ in astrocytes in primary culture

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Astrocytes protect neurons from oxidative stress and inflammation induced by the $A\beta_{1-42}$ peptide. Aspirin is commonly used as an antiplatelet and anti-inflammatory drug in clinical practice. However, its effects in the presence of the $A\beta_{1-42}$ peptide are poorly understood. We analysed the effects of aspirin, at concentrations corresponding to those achieved in clinical practice, on astrocytes in primary culture in the presence or absence of the $A\beta_{1-42}$ peptide. Specifically, we determined the effects of aspirin (10^{-7} M) on the inflammatory and oxidative processes caused by the toxic peptide in astrocytes in primary culture.
After the addition of $A\beta_{1-42}$, we detected an increase in pro-inflammatory mediators (IL-β and TNF-α) and in the expression of the NF-κB protein, with a decrease in the expression of the anti-inflammatory protein PPAR-γ compared to the control values. Furthermore, the presence of $A\beta_{1-42}$ increased the expression of COX-2 and iNOS and decreased the antioxidant proteins Cu/Zn-SOD and Mn-SOD. In the presence of the toxic peptide, the addition of aspirin (10^{-7} M) decreased pro-inflammatory mediators (IL-β and TNF-α) and the expression of the NF-κB protein, increasing the anti-inflammatory protein PPAR-γ and prevented the decrease in the expression of Cu/Zn-SOD and Mn-SOD induced by the $A\beta_{1-42}$ peptide. On the other hand, aspirin avoided the elevation of the expression of COX-2 and iNOS induced by the $A\beta_{1-42}$ peptide.
In conclusion, aspirin at therapeutic concentrations prevents the deleterious inflammatory and oxidative effects induced by the presence of the toxic peptide $A\beta_{1-42}$ in astrocytes in primary culture. The use of low doses of aspirin could be useful for the control of inflammation and oxidative stress generated by the presence of the $A\beta_{1-42}$ peptide in Alzheimer's disease.

NC56
White-wine pomace product modulates oxidative stress and inflammation in endothelial dysfunction through NF-κB

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The endothelial dysfunction is associated with the development of atherosclerosis, hypertension and cardiovascular events. Oxidative stress and inflammation are connected in the endothelial dysfunction. RONS upregulated adhesion molecules through NF-κB stimulation and decreased NO bioavailability. According to various studies, wine pomace products can improve vascular function and reduce endothelial dysfunction. This study investigated the antiinflammatory effects against endothelial dysfunction of two digested fractions of white wine pomace product (wWPP) in hyperglycemic endothelial cell line EA.hy926. To elucidated this, we analized the NF-κB pathway and its impact on the nitric oxide and RONS levels. The results revealed that in vitro digested fractions of wWPP showed anti-inflammatory actions due down-regulation of the NF-κB pathway, thereby affecting cell viability and the NO/RONS balance. These data evidence of the mechanisms that activated the protective effects of wWPP and the potential benefits in cardiovascular diseases. The authors thank the financial support of Ministry of Science, Innovation and Universities, Spanish State Research Agency and European Regional Development Fund (Project PGC2018-097113-B-I00).

NC57
Modulation of mitochondrial, autophagic, and nutrient sensing pathways markers in liver and skeletal muscle by overexpression of the redox enzyme NADH-cytochrome b_5 reductase-3 in male and female mice

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Existing data on the differential susceptibility to diseases or a different effect of drugs in men and women demonstrates the existence of sexual dimorphism, which must be taken into account when designing research strategies. Differences between males and females have been also shown for model organisms, which can affect a multitude of processes. Including both sexes in the design of the studies is thus important to gain a greater relevance of the research from a biomedical point of view. Previous studies developed in our group have been focused on the redox enzyme NADH-cytochrome b_5 reductase-3 (CYB5R3) as a new pro-longevity gene. We have shown that CYB5R3 expression contributes to maintain respiratory metabolism, protects against oxidative stress, and prevents cellular senescence in male mice overexpressing CYB5R3. Since our previous investigation has demonstrated that the outcome of an antiaging intervention as calorie restriction is strongly influenced by sex, the main objective of our work was to study the potential existence of sexual dimorphism in several markers of biochemical pathways related with the hallmarks of aging, such as the levels of mitochondrial complexes, autophagy markers and nutritional sensors. Studies were carried out in skeletal muscle and liver samples obtained from CYB5R3 transgenic and control mice of both sexes in a C57BL/6 background at three months of age. The effects of CYB5R3 overexpression on mitochondrial complexes, autophagy markers and nutritional sensors showed sexual dimorphism that was also tissue specific. The increased autophagic flow in liver and skeletal muscle seen in CYB5R3 transgenic males, did not occur in females. On the other hand, the increase in hepatic mitochondrial complexes was more noticeable in CYB5R3 transgenic females. These observations reinforce the need for further in-depth studies, including longevity studies, focused on animals of both sexes.
Keywords: CYB5R3, sexual dimorphism, skeletal muscle, liver

NC58
New antioxidant soy-derived bioactive peptides released through simulated gastrointestinal digestion modulate Keap1/Nrf2 pathway response

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Food-derived bioactive peptides are a group of molecules with health-promoting effects on many body functions. They are protein fragments of 2-20 amino acids that can be released by various mechanisms, such as gastrointestinal digestion

and food processing due to microbial fermentation. Bioactive peptides are inactive in the native structure of the food proteins but, once hydrolysed from the macromolecules, they can exert different effects on the body including antihypertensive, opioid and antioxidant activity.
In this work, fermented soy products were digested *in vitro* in order to improve the release of bioactive peptides and then six peptide-enriched fractions were extracted from the food matrix, purified and analysed *in vitro* and in a cellular model, using Caco-2 cells, in order to select the most antioxidant fraction. Subsequently, the sequence of bioactive peptides was identified by LC-MS/MS analysis. In order to assess the molecular mechanism of action of the bioactive peptides, the possible interaction of these molecules with Keap1 protein was evaluated by a molecular docking approach. This factor is one of the key proteins of Keap1/Nrf2 pathway, a major system involved in redox homeostasis. The peptides that showed a high score of interaction with Keap1 were selected and analysed in Caco-2 cells. Firstly, the selected peptides were not cytotoxic, protected cells toward oxidative stress and decreased ROS production in cells treated with TbOOH. Moreover, it was observed that peptides, showing the highest interaction score with Keap1, increased nuclear levels of Nrf2. This observation suggested that the peptides were able to activate Keap1/Nrf2 pathway, as an overexpression of antioxidant and phase II enzymes was observed in cells treated with these peptides. Therefore, we can conclude that the interaction observed *in silico* was confirmed by the results obtained in the cellular model.

NC59
Reactive Oxygen Species as Unifying Factor in Insulin Resistance

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Insulin Resistance, a pathophysiological state defined as a loss of insulin sensitivity by numerous body tissues, is one of the earliest manifestations of Type II Diabetes. It is characterised on the molecular level as the inability of the insulin signalling cascade to mediate the translocation of glucose transporter GLUT4 to the plasma membrane. A variety of cellular perturbations have been previously associated with the insulin-resistant phenotype, including increased levels of lipid ceramide, reduced levels of mitochondrial electron carrier Coenzyme Q, mitochondrial stress and an elevation in cellular Reactive Oxygen Species (ROS). In our ongoing study, we posit that ROS sits at the intersection of these perturbations and holds the potential to act as a driver of insulin resistance downstream, culminating in defective GLUT4 membrane trafficking.
We utilise a suite of new, sensitive cellular models to examine the identity, localisation and kinetics of ROS production in Insulin resistance. These models differ from the older, more harsh means of inducing insulin resistance by limiting dose and exposure of cells to insults, in turn allowing for a stronger differentiation between the consequences and causes of insulin resistance. Here we demonstrate that acute Insulin stimulation produces an oxidative shift in the cellular redox state, and this shift is exacerbated within the aforementioned models of insulin resistance. Additionally, our work implies a role for Reactive Nitrogen Species alongside Reactive Oxygen Species within this insulin-mediated shift in redox state. We hope that these data form the foundation for a more streamlined model of insulin resistance and will one day progress to therapeutic applications in the treatment of Insulin Resistance and Type II Diabetes within a clinical setting.
Keywords: Insulin Resistance, ROS, Mitochondria, Insulin, GLUT4

NC60
Metformin reduces mitochondrial ROS levels and improves mitochondrial mass, membrane potential and respiratory complexes in leukocytes of type 2 diabetic subjects

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Type 2 diabetes (T2D) is a chronic inflammatory-based disease, which can lead to cardiovascular damage. Metformin is a first-line hypoglycaemic drug for T2D that acts mainly by lowering glucose levels. It has also been shown to have mitochondrial effects, but the underlying mechanism is yet to be elucidated. In the present study, we describe how T2D can affect mitochondrial function in peripheral blood leukocytes from T2D patients, and how metformin can modulate this effect.
Peripheral blood was extracted from 80 T2D patients and 80 healthy volunteers. Among the T2D patients, 40 had been treated with 1700 mg/day metformin for at least 1 year. Peripheral blood mononuclear cells (PBMC) were isolated fol-

lowing a ficoll density protocol and an erythrocyte lysis and were subsequently employed in our experiments. Mitochondrial mass, mitochondrial membrane potential and reactive oxygen species (ROS) content were analysed by flow cytometry with Mitotracker green, tetramethylrhodamine and MitoSox fluorescence, respectively. Protein analysis was performed by SDS-PAGE western blot.
We observed that mitochondrial mass and mitochondrial membrane potential were lower in leukocytes from T2D patients than in those of healthy controls, and that this effect was reversed in the presence of metformin. In parallel, mitochondrial ROS levels were higher in T2D leukocytes, while metformin reduced ROS levels. In relation to protein levels in mitochondrial complexes, leukocytes from the T2D patients displayed lower complex I, II, III and V protein content, while no changes were observed with respect to complex IV. Metformin treatment returned all the affected complexes to control levels.
In conclusion, we demonstrate that metformin modulates mitochondrial function and structure in T2D patients.
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NC61
Type 1 diabetic patients exhibit enhanced leukocyte-endothelium interaction and mitochondrial alterations

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Cardiovascular complications are the leading cause of death among patients with type 1 diabetes (TD1). Hyperglycaemia is one of the principal risk factors for developing cardiovascular diseases (CVD), mainly through the induction of oxidative stress. The overproduction of reactive oxygen species (ROS) promotes atherosclerosis by enhancing inflammation. We evaluated whether T1D patients present alterations in leukocyte-endothelium interaction, oxidative stress, and inflammatory parameters.
We recruited forty controls and forty-five patients with T1D. Anthropometric measurements were performed and blood samples obtained for biochemical determination and molecular analysis in all the subjects. Levels of leukocyte adhesion molecules (Selectin P, VCAM1 and ICAM1), proinflammatory cytokines (TNF-α and IL-6) and myeloperoxidase were analysed in the patients' serum. Interactions between patients' leukocytes and endothelial cells were evaluated using an *ex vivo* model. In leukocytes, we assessed mitochondrial function and determined several oxidative stress parameters with fluorescent probes: DCFH-DA for total ROS production, MitoSOX for mitochondrial ROS production and TMRM for mitochondrial membrane potential.
As expected, T1D patients exhibited higher levels of glucose and Hba1c-DCCT than controls (both $p<0.001$), and enhanced leukocyte-endothelium interactions, with reduced PMN rolling velocity ($p<0.001$) and PMN rolling flux ($p<0.01$) and greater PMN adhesion ($p<0.001$). In parallel with these results, serum from T1D patients presented higher levels of leukocyte adhesion molecules Selectin P ($p<0.05$), VCAM1 ($p<0.01$), and ICAM1 ($p<0.001$). Moreover, serum levels of TNF-α ($p<0.01$) and myeloperoxidase ($p<0.05$), but not IL-6, were higher in T1D patients. Finally, T1D leukocytes presented mitochondrial alterations, with enhanced total and mitochondrial ROS production (both, $p<0.05$) and increased mitochondrial membrane potential ($p<0.05$).
In conclusion, mitochondrial alterations, increased leukocyte-endothelium interactions, and oxidative stress may be related to the development of CVD in T1D.
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NC62
Resveratrol effects on SIRT1-SIRT3-SOD2 axis in high-glucose-challenged HUVECs

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Uncontrolled accumulation of methylglyoxal and reactive oxygen species occurs in hyperglycemia-induced endothelial dysfunction associated with diabetes. In our previous publication, we have demonstrated the ability of resveratrol (RSV) to protect high-gluocse (HG)-challenged endothelial cells mainly by activating the sirtuin 1 (SIRT1, a NAD^+-dependent deacetylase) - glyoxalase 1 pathway, enhancing antiglycative and antioxidant defences and abolishing the HG-dependent peroxidative and glycative damages (1).
Since mitochondria are a hub of oxidative stress, the aim of the present study is to investigate the role of SIRT1 on mitochondrial response to HG in endothelial cells. Our experimental model consists of primary human umbilical vein endothelial cells (HUVECs) undergoing a 24-h treatment with HG, with or without RSV and EX527 (a SIRT1 inhibitor). We evaluated the sirtuin 3 (SIRT3) mRNA levels, through RT-PCR, and SIRT3 protein levels as well as acetyl-superoxide dismutase 2 (ac-SOD2) over total SOD2, through western immunoblotting.
Our data indicate that HG treatment induce a decrease in SIRT3 protein and an increase of ac-SOD2. RSV is able to restore SIRT3 levels and reduce the acetylation levels of SOD2, suggesting that RSV may improve the mitochondrial antioxidant milieu through the activation of SIRT3/SOD2 axis. Surprisingly, when SIRT1 is inhibited both the acetylation levels of SOD2 and SIRT3 levels do not change. These findings suggest that the effect of RSV on SOD2 acetylation seems not to be SIRT1-dependent.
Our future work will focus on the investigation of mitochondrial respiratory function and morphology in order to better clarify how RSV and SIRT1 may protect the mitochondrial environment of HG-challenged HUVECs.
Reference:
Santini SJ, Cordone V, Mijit M, et al. SIRT1-Dependent Upregulation of Antiglycative Defense in HUVECs Is Essential for Resveratrol Protection against High Glucose Stress. *Antioxidants (Basel)*. 2019;8(9):346. Published 2019 Sep 1. doi:10.3390/antiox8090346

NC63
Involvement of Akt-p38-MAPK/Nrf2 pathway in prevention of endothelial damage by white-wine pomace product

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Hyperglycemia constitutes one of the main characteristics of diabetes, obesity and other cardiovascular diseases. This risk factor increases the oxidative stress and causes tissue damage through several mechanisms, including alterations in several signaling pathways. The main aim of this study was to investigate the mechanism involved in the preventive effect against endothelial oxidative damage of bioavailable fractions obtained from white wine pomace product (wWPP)**.** The Nrf2/ARE pathway is involved in the increase of oxidative stress in hyperglycemic endothelial cells (EA hy926). The treatment with bioavailable wWPP showed a stimulatory effect on Akt-p38-MAPK/Nrf2 pathway, restored the balanced redox environment and reduces cell membrane damage. These results confirm the promising healthy properties of bioavailable fraction of wWPP in cardiovascular diseases. The authors thank the financial support of Ministry of Science, Innovation and Universities, Spanish State Research Agency and European Regional Development Fund (Project PGC2018-097113-B-I00).

NC64
The research about antioxidant and anti-obesity effects of tocotrienols in high-fat diet-treated mice

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As the elderly people is increasing all over the world, many social problems are occurred such as the increasing caregiver burden and medical costs. The main reason why elderly people need to care is the onset of dementia. About 60 % of dementia is occupied by Alzheimer's disease (AD). Recently, some reports have indicated that obesity raise a risk of AD, however, the mechanisms have not been elucidated in detail. It has been well known that oxidative damage in living tissues is strongly involved in the AD onset. Additionally, oxidative stress is accelerated in obesity. From the above, many researchers are investigating the anti-obesity effects of natural compounds such as caffeine and ginger.
We are also focusing on the anti-obesity effects of tocotrienols (T3s), which are one kind of vitamin E. The most famous function of T3s is antioxidant. However, these beneficial effects of T3s in obese model mice have not been elucidated completely.

From these backgrounds, the purpose of this study is to assess the anti-obesity effects of T3s on high-fat diet treated mice, to measure the changes in cognitive function and antioxidative defense systems in brain. As the results, T3s significantly inhibited the body weight gain and lipid droplets synthesis in liver. Additionally, treatment with T3s significantly altered the cognitive function in the Morris water maze test. We want to show these detailed-results in our presentation.
Key words; Anti-obesity, antioxidants, cognitive dysfunction

NC65
Dietary (-)-epicatechin mitigates high fat-induced apoptosis, oxidative and endoplasmic reticulum stress in pancreatic β-cells both *in vivo* and *in vitro*

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In the past decades type 2 diabetes (T2D) is becoming a major public health concern worldwide. β-cell apoptosis is central to the pathophysiology of T2D. Oxidative and endoplasmic reticulum (ER) stress, both associated with consumption of Western-style diets, underlie β-cell apoptosis. We previously showed that, the flavan-3-ol (-)-epicatechin (EC) improves insulin sensitivity in part by mitigating oxidative/ER stress in the liver and adipose tissue from mice fed a high fat (HF) diet. The aim of this study was to investigate, both *in vivo* (HF-fed mice) and *in vitro* (INS-1 cells) the capacity of EC to mitigate HF diet-induced oxidative/ER stress and the consequent β-cell apoptosis. *In vivo*, the activation of: i) the insulin pathway, and ii) components of the three UPR branches (PERK, ATF6, XBP-1, JNK) and iii) oxidative stress (NOX1, NOX4, 4-HNE) were measured in mice fed for 15 w: a control diet (C), and a high fat diet without (HF) or with EC (20 mg EC/kg body weight). The response to insulin was impaired in the pancreas of HF-fed mice, which was mitigated by EC supplementation. All the three UPR branches were activated in the pancreas from HF-fed animals. EC supplementation decreased the phosphorylation of PERK and JNK, and the levels of spliced XBP-1 and ATF-6. Moreover, EC supplementation prevented HF diet-associated pancreatic triglycerides deposition and enlargement of islets. *In vitro*, INS-1 β-cells, treated with palmitate (1 mM) caused ER stress (activation of ATF6, IRE1α and PERK), increased expression of NOX1 and NOX4, protein carbonylation, and activation of the redox-sensitive NF-κB pathway. INS-1 cell apoptosis was evidenced by caspase 3 activation. EC (0.5-1 μM) mitigated or prevented all the adverse effects of palmitate in INS-1 cells. In summary, consumption of EC-rich diets, together with an improved lifestyle, could help mitigate obesity associated β-cell dysfunction and T2D progression.

NC66
Supplementation with anthocyanins reverts established endotoxemia in high fat-fed mice through the regulation of colonic physiology by modulating redox signaling

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Consumption of high fat diets (HFD) can impair intestinal barrier integrity, leading to endotoxemia and associated unhealthy conditions. We previously observed that 15-w consumption of a HFD together with an anthocyanin (cyanidin and delphinidin glycosides)-rich blend (ACB), prevented HFD-induced alterations in intestinal monolayer integrity, endotoxemia and dysbiosis in mice. The current study investigated the effects of a short-term ACB supplementation on HFD-induced alterations of colonic physiology and endotoxemia. Six-week-old C57BL/6J male mice were fed control or high fat diets for 4 w. Then, mice on each group were subdivided in two groups that either continued on control and high fat diets, or were supplemented with 40 mg ACB/kg BW for the subsequent 4 w. Consumption of the HFD for 8 w caused endotoxemia evaluated as plasma lipopolysaccharides (LPS), increases in circulating LPS-binding protein levels and alterations in parameters of colonic function. These events were mitigated by 4-w supplementation with ACB. Thus, in the colon, consumption of the HFD caused: i) an increase in TLR4 expression; ii) a decrease in tight junction protein levels, i.e. occludin, ZO-1 and claudin-1; iii) an increased expression of NADPH oxidase NOX1 and iv) the inhibition of redox sensitive pathways, i.e. NF-κB and ERK1/2. HFD consumption affected parameters of goblet cell differentiation and function. ACB acted mitigating HFD-downregulation of markers (mRNA levels) of goblet cell differentiation (Klf4) and mucin production (Muc2). ACB also reverted HFD-induced reduction of Tff3 mRNA levels, a key mediator of the intestinal mucosa healing process. The latter effects could be in part explained by ACB's capacity to downregulate the PI3K/Akt pathway. In fact, ACB decreased HFD-induced PI3K upregulation and downstream activation of Akt. Our

findings show that select anthocyanidins, particularly cyanidin and delphinidin, could mitigate HFD-induced alterations in colon physiology and the associated endotoxemia in part through the modulation of redox signaling.

NC67
Phenolic compounds from *Iris hungarica* as potential anti-inflammatory agents

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Background: Plant herbs can deliver significant health benefits as anti-inflammatory and antioxidant agents. A variety of chemical constituents such as coumarins, carotenoids, flavonoids, steroids, fatty acids, stilbenes, and terpenoids are isolated from plant origin which significantly shows anti-inflammatory activities in different models. Hence, this approach for the treatment of inflammatory diseases by herbal drugs has a keen interest. *Iris* genus (*Iridaceae*) comprise of plant species that widely grow and are cultivated in European and Asian countries. Rhizomes of some *Iris* species contain flavonoids such as iridin, iriline A, irisone B. *Iris* leaves contain many flavonoids such as irilin, iristectorigenins, tectorigenin. *Iris* flowers contain also flavonoids and proanthocyanidin.
Aim: In this study, sixteen phytochemical compounds were isolated from rhizomes of *Iris hungarica* by column chromatography and evaluated by the anti-inflammatory activity. Isolation was carried out by using chromatographic techniques and the structures were elucidated by 1D and 2D NMR spectroscopy.
Material and Methods**:** Ethylacetate fraction of ethanolic extract of *I. hungarica* rhizomes was separated using silica gel column chromatography eluted with chloroform: ethanol (in different proportion) as solvent system. The isolated compounds were subjected to further spectral analysis (MS, ^{1}H-NMR and ^{13}C-NMR). The anti-inflammatory activity of compounds was evaluated by measuring superoxide anion generation and elastase release in human neutrophils.
Results and Discussion: Known isoflavones irisolidone, kikkalidone, irigenin, irisolone, irilone, genistein, daidzein, ononin, formononetin, as well as xanthone mangiferin and 2′-hydroxy-4′-glucosyl chalcon were isolated for the first time from *I. hungarica* rhizomes.
Conclusion**:** The results of the anti-inflammatory activity of the compounds - daidzein, formononetin, and 2′-hydroxy-4′-glucosyl chalcon showed moderate activity, as well as other compounds had a more pronounced effect.

NC68
NADPH oxidase 4 (Nox4) deletion accelerates liver regeneration in mice

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Liver is a unique organ in displaying a reparative and regenerative response after acute/chronic damage or partial hepatectomy, when all the cell types must proliferate to re-establish the liver mass. The NADPH oxidase NOX4 mediates Transforming Growth Factor-beta (TGF-β) actions, including apoptosis in hepatocytes and activation of stellate cells to myofibroblasts. Aim of this work was to analyze the impact of NOX4 in liver regeneration by using two mouse models where *Nox4* was deleted: 1) general deletion of *Nox4* (NOX4-/-) and 2) hepatocyte-specific deletion of *Nox4* (NOX4hepKO). Liver regeneration was analyzed after 2/3 partial hepatectomy (PH). Results indicated an earlier recovery of the liver-to-body weight ratio in both NOX4-/- and NOX4hepKO mice and an increased survival, when compared to corresponding WT mice. The regenerative hepatocellular fat accumulation and the parenchyma organization

recovered faster in NOX4 deleted livers. Hepatocyte proliferation, analyzed by Ki67 and phospho-Histone3 immunohistochemistry, was accelerated and increased in NOX4 deleted mice, coincident with an earlier and increased *Myc* expression. Primary hepatocytes isolated from NOX4 deleted mice showed higher proliferative capacity and increased expression of *Myc* and different cyclins in response to serum. Transcriptomic analysis through RNA-seq revealed significant changes after PH in NOX4-/- mice, and support a relevant role for *Myc* in a node of regulation of proliferation-related genes. Interestingly, RNA-seq also revealed changes in the expression of genes related to activation of the TGF-β pathway. In fact, levels of active TGF-β1, phosphorylation of Smads and levels of its target p21 were lower at 24 h in NOX4 deleted mice. Nox4 did not appear to be essential for the termination of liver regeneration *in vivo*, neither for the *in vitro* hepatocyte response to TGF-β1 in terms of growth inhibition, which suggest its potential as therapeutic target to improve liver regeneration, without adverse effects.

NC69
A novel nutraceuticals mixture improves oxidative stress by reprogramming mitochondrial bioenergetics in a non-alcoholic fatty liver disease model

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Non-alcoholic fatty liver disease (NAFLD) is the leading cause of liver disease globally, with very scarce treatment options. The underlying mechanisms of NAFLD progression to non-alcoholic steatohepatitis (NASH) and cirrhosis are not understood yet. Several factors contribute to NAFLD pathogenesis such as insulin resistance, lipid overload, microbiota; however recent studies reported redox biology impairment as the cornerstone of NAFLD progression. Though a very little is known about micronutrients role in NAFLD, recent studies reported the potential antioxidant properties of vitamins and other elements.
We investigated the role of dietary supplementation with FLINAX, a novel mixture of nutraceuticals (i.e. vitamin E, vitamin D3, olive dry-extract, cinnamon dry-extract and fish oil) in a model of NAFLD characterised by oxidative stress and mitochondrial function impairment.
Wistar rats were fed with high fat-high cholesterol diet for 4 weeks to induce steatohepatitis. Following, a group was fed with chow-diet supplemented with 2% FLINAX, while chow-diet only served as control. Liver tissues and isolated mitochondria were used for steatosis, oxidative stress and mitochondrial function analysis.
Use of FLINAX supplementation significantly reduced steatosis and hepatic triglycerides content. The expression studies indicated that FLINAX enhanced fatty acids oxidation (FAO) as *CPT1A* and *CPT2* resulted up-regulated. Lower levels of lipoperoxidation markers (i.e. HNE- and MDA-protein adducts) were detectable in FLINAX-treated rats. Accordingly, the total amount of mitochondrial oxidised proteins was lower, and the enzymatic activity of mitochondrial SOD was not required in FLINAX-supplemented rats compared to controls. Intriguingly, FLINAX enhanced the activity of mitochondrial respiratory chain (RC) complex I, II, III and of ATP-synthase (complex V). Accordingly, the peroxide production from pyruvate/malate (complex I) and succinate (complex II) was dampened by FLINAX-treatment.
Therefore, the dietary supplementation with FLINAX fuelled the RC activity and contrasted ROS formation, ameliorating mitochondrial function and enhancing FAO with the consequent reduction of lipid storage.

NC70
Enniatins effect in rat liver through a proteomic approach

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Enniatins (ENNs) are hexadepsipeptides produced by *Fusarium* fungi which can act as ionophores, disturbing membranes homeostasis. In this study, a proteomic analysis to determine acute response of rat's liver to ENs exposure at different concentrations was carried out. A total of 14 female Wistar rats were employed divided in three groups. Five of the treated ones were intoxicated with medium concentrations: EN A 256, ENN A1 353, ENN B 540, ENN B1 296 µg/mL; and other five with the higher ones: ENN A 513, ENN A1 706, ENN B 1021, ENN B1 593 µg/mL during 8 hours of exposure. Protein extraction was performed using 10 mg of powdered liver tissue. Afterwards, tryptic digested samples were dried on a vacuum concentrator and eluted to a final concentration of 100 µg/µL. Peptides were analysed using a LC system

coupled with quadrupole time of flight (Q-TOF) and the obtained chromatograms were aligned with Mass Hunter Professional software (Agilent). Proteins identification was carried out by Spectrum Mill software and statistically filtered by abundance reporting a total of 57 differentially expressed proteins in both medium and high treated animals when compared to the control. In terms of abundance, carbamoyl phosphate synthase-1 had the highest expression level whereas actin-1 had the lowest one. Gene ontology analysis revealed acetylation, nucleotide phosphate-binding region: NAD and catalytic activity as the most represented terms in the bioinformatics analysis. Moreover, 13 of these proteins were found in the mitochondrion and 12 were related to oxidoreductase activity. Considering the oxidoreductase and the antioxidant activities, four proteins participate in both processes: Superoxide dismutase 1, Peroxiredoxin 4, Hemoglobin alpha and beta. Regarding Reactome results, metabolism was both the most significant pathway, followed by tricarboxylic acid and electron transport chain.
Keywords**:** mycotoxin, proteomics, Q-TOF, mitochondrion.
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NC71
Oxidative phosphorylation provides stress resistance in non-proliferating cells

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Mitochondrial oxidative phosphorylation (OXPHOS) generates ATP, but also has other important roles that are ATP-independent. In proliferating cells OXPHOS is required for pyrimidine synthesis, but in non-proliferating cells its ATP-unrelated functions have not been defined. Here we demonstrate that in non-proliferating post-mitotic cells OXPHOS maintains stress resistance. OXPHOS deficiency leads to suppression of autophagy and increased sensitivity to oxidative stress in vitro and in TFAM knockout mice *in vivo*, and attenuation of autophagy in OXPHOS-functional background phenocopies the effects of OXPHOS deficiency. Mechanistically, the autophagy/stress response defect is independent of transcriptional control, AMPK/mTOR1/ULK1 signaling or upregulation of NADH. Instead, absence of OXPHOS-derived ROS in OXPHOS-deficient cells leads to excessive activation of ATG4, a redox-sensitive suppressor of autophagy. We propose that maintenance of stress resistance via the ROS-ATG4 axis is an important function of mitochondrial respiration in non-proliferating cells that may have consequences for longevity and cancer therapy.
Keywords: Mitochondria, autophagy, oxidative phosphorylation, electron transport chain, reactive oxygen species

NC72
Simvastatin prevents IL-1β potentiation of bradykinin-induced microvascular permeability in intact skeletal muscle: involvement of NADPH oxidase and reactive oxygen species

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Statins have been reported to reduce microvascular permeability and edema formation in animal models and clinical studies. Bradykinin (Bk) induced increases in cerebral microvascular permeability are potentiated by IL-1β, yet protection by statins against such hyperpermeability has not been investigated. We have examined the effects of pre-treatment of rats with simvastatin (5mg.kg^{-1}, i.p.) on FITC-albumin permeability of post-capillary venules in rat cremaster muscle. Bradykinin (1-100nM) induced permeability increases were unaffected by L-NAME but abrogated by SOD and catalase. Acute perfusion of the cremaster muscle with IL-1β (30pM, 10min), followed by bradykinin, caused a leftward shift of the bradykinin dose–response curve. IL-1β potentiation of bradykinin induced permeability was prevented by the NADPH oxidase inhibitor apocynin (1μM). Simvastatin pre-treatment (24 h) prevented IL-1β potentiation of bradykinin permeability responses, which were not affected by inhibition of heme oxygenase-1 with tin protoporphyrin IX (SnPP). Our findings suggest that simvastatin prevents microvascular hyperpermeability induced by IL-1β and bradykinin via inhibition of NADPH oxidase and inhibition of reactive oxygen species generation, highlighting the potential of statins in the prevention and treatment of patients predisposed to inflammatory diseases.
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NC73
S-glutathionylation of the Na^+-K^+ pump is a novel redox mechanism in preeclampsia

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Reduced Na^+-K^+ pump activity is widely reported in preeclampsia. We have identified glutathionylation, a reversible posttranslational redox modification, of the β1 Na^+-K^+ pump subunit (GSS-b1) as a regulatory mechanism of pump activity. We hypothesized that GSS-b1 may occur in PE placentas and participate in pump dysfunction.
The GSS-b1 was investigated in placentas from eleven women with PE and in eleven with normal pregnancies. Protein expression of the b1 subunit was unchanged in placenta from women with preeclampsia versus those with normotensive pregnancies. Immunofluorescence and western-blot experiments carried out on β1 Na^+-K^+ pump subunit immunoprecipitates, showed a high level of GSS-b1 in PE placentas, mostly reversed by dithiotreitol (DTT), thus revealing of S-glutathionylation. This was subsequently linked with a decrease in α1/β1 subunits coimmunoprecipitation. The cytosolic $p47^{phox}$ NADPH oxidase subunit and its coimmunoprecipitation with the α1 subunit of Na^+-K^+ pump was increased in preeclamptic placentas, thus indicative of NADPH oxidase-dependent pump inhibition.
The high level of β1 pump subunit glutathionylation in preeclamptic placentas provides new insights in the mechanism of Na^+-K^+ pump dysfunction in this disease.
Keywords: Na^+-K^+ pump; Glutathionylation; Preeclampsia; Oxidative stress; NADPH oxidase; Preeclampsia

NC74
Higher levels of eryptosis and erythrocyte adhesion to vascular endothelium in hypercholesterolemic subjects regardless statin therapy: a pilot study

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Background: Eryptosis can be triggered by oxidative stress inducing externalization of phosphatidylserine (PS) out of the membrane which facilitates the eryptotic cell binding to endothelial cells, potentially leading to atherosclerosis. Accordingly, cholesterol oxides, in hypercholesterolemia-relevant proportion, have shown an *ex vivo* pro-eryptotic activity in erythrocytes of healthy individuals. Aim: To determine the levels of eryptosis and interactions of eryptotic erythrocytes with endothelial cells in hypercholesterolemic patients either non-medicated or medicated, compared with healthy subjects.
Methods: 56 subjects clustered in three groups (control (*n*=20), hypercholesterolemic non-treated (HCNT) (*n*=15) and statin-treated (HCT) (*n*=21)) were enrolled in this cross-sectional study. Biochemical parameters were determined with validated and standard methods. PS exposure was estimated from annexin-V-binding, cell volume from forward scatter and GSH from CMFDA fluorescence by flow cytometry. The erythrocyte-endothelium cells adhesion assay was performed by using the parallel-plate flow chamber technique with human umbilical vein endothelial cells (HUVECs).
Results: Higher PS externalization and adhesion of eryptotic erythrocytes to vascular endothelium was found in hypercholesterolemic subjects, independently of statin treatment, compared to the control group (PS externalization: control: 0.99±0.34%; HCNT: 1.23±0.31%; HCT: 1.39±0.61%. Adhesion: control: 5.5±0.8 cells/mm^2; HCNT: 11.7±1.8 cells/mm^2; HCT: 13.4±1.6 cells/mm^2). The presence of systemic inflammation (high hsCRP values), high ApoB (HCNT) or low ApoA (HCT) values together with a trend of higher oxidative stress (low GSH) could be the inducers of eryptosis in hypercholesterolemic patients.
Conclusion: High levels of eryptosis and adhesion of eryptotic erythrocytes to endothelial cells, could be indicators of risk of cardiovascular disease with possible development of atherosclerosis and microcirculation problems in hypercholesterolemic patients, independently of statin therapy.
Keywords: Hypercholesterolemia, eryptosis, phosphatidylserine exposure, erythrocyte-endothelium interactions, oxidative stress.
Acknowledgements: This study was financed by the VLC-BIOCLINIC 2017 Subprogram A of the University of Valencia and Institute of Health Research (INCLIVA) (Valencia, Spain).

NC75
Early reductive stress followed by a late onset oxidative stress in acute myocardial infarction

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Introduction: The idea that the cells might suffer from reductive rather than oxidative stress and that such stress may be relevant in pathophysiology has gained momentum.
Aim: We aimed at studying markers of oxidative stress and damage as well as the expression of antioxidant enzymes in a swine model of acute myocardial infarction (AMI) followed by reperfusion.
Results and Discussion: We found an increase in the GSH to GSSG ratio, a decrease in protein glutathionylation and a decrease in p38 MAPK phosphorylation after 90 minutes of ischaemia in heart samples. It was accompanied by an increase in the expression of Thioredoxin (TrX) and Peroxiredoxin (PrX) and a decrease in the expression of Glutathione Peroxidase (Gpx). One week after reperfusion an increase in lipid peroxidation, protein glutathionylation and protein carbonylation was accompanied by a massive increase in the expression of TrX, PrX, GPx, Glutathione Reductase (GR) and Glucose 6 Phosphate Dehydrogenase (G6PD). These increments seemed to be Nrf-2 independent and most of them were maintained in the heart samples one month after reperfusion.
Conclusion**:** We have found an early reductive stress followed by a late onset oxidative stress in AMI followed by reperfusion.

NC76
Abrogating mitochondrial ROS in neurons or astrocytes reveals cell-specific impact on mouse behaviour

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Cells naturally produce mitochondrial reactive oxygen species (mROS), but the in vivo pathophysiological significance has long remained controversial. Within the brain, astrocytes produce mROS at one order of magnitude faster than neurons. Recently, we demonstrated that these endogenous high levels of astrocytic mROS are modulating brain metabolism and behaviour. However, whether mROS specifically produced by neurons are influencing on behaviour in living mice is elusive. To address this, we engineered genetically modified mice expressing a mitochondrial version of catalase to selectively knockdown mROS cell-type specifically in vivo. We compared the behavioural phenotype after reducing neuronal mROS in basal conditions with that of astrocyte-specific mROS downregulation in vivo. Under healthy conditions, in contrast of the observed deleterious effects by abolishing mROS in astrocytes, our data show lack of significant behavioural impairment by downmodulating mROS in neurons. However, under the pathological challenge caused by the administration of the pro-oxidant neurotoxin, 3-nitropropionic acid (3-NP), we observed that motor discoordination was abolished by preventing the increase of neuronal, but not astrocytic, mROS production. These results indicate that the pathophysiological role(s) of mROS should be investigated by dissecting out the specific cell type where they are produced. We believe that this study helps understanding the long-lasting controversy around the protective versus deleterious role(s) of brain mROS, and suggests that mROS should be targeted in a cell-specific manner in order to ascertain their physiological functions and impact on behaviour.

NC77
Establishment of analytical method to detect Coenzyme Q10 level in mitochondrial supercomplex

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Introduction: Recent studies reveal that the mitochondrial electron transport chain proteins are present by forming a supercomplex rather than being independent of each other. Coenzyme Q10 (CoQ10) is an essential lipid in the mitochondrial respiratory chain and this lipid is included in supercomplex. CoQ10 also existed outside of mitochondria supercomplexes. CoQ10 also presence in other membranes such as plasma membrane and nuclear membrane. Levels of CoQ10 in cell are payed much attention because its level is reduced in aging and various diseases. However, whether the levels of CoQ10 in supercomplexes are also reduced in aging and in various diseases or not, is still under debate. In this study, we tried to establish a CoQ quantitative method which is included in the mitochondrial respiratory chain supercomplex.
Methods: Mitochondria were extracted by centrifugation from the cultured cells and mouse livers. A mitochondrial membrane sample was separated by blue native electrophoresis technique (BN-PAGE). Each respiratory protein was detected by using In-gel enzyme activity assay and Western blotting. The gels were cut in 3 mm slices. CoQ was extracted from these slices by using hexane. CoQ was detected by HPLC-ECD.
Results and Discussion: The presence of the mitochondrial respiratory chain supercomplex was confirmed by BN-PAGE and in-gel enzyme activity assay. Western blotting analysis also exhibited the existence of supercomplexes. CoQ was detected in the gel slice with supercomplex proteins, showing that CoQ is existed in supercomplex. Administration of 4-nitrobenzoate, inhibitor of CoQ biosynthesis, decreased CoQ level both in supercomplex and other places. It is expected to analysis CoQ level in supercomplex in various samples by using this method.
Keywords: Coenzyme Q10, Mitochondria, Respiratory chain

NC78
Mitochondrial ROS contribute to neuronal ceroid lipofuscinosis pathogenesis

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Neuronal ceroid lipofuscinoses (NCLs), known as Batten disease, are the most common of the rare neurodegenerative disorders in children. These disorders are grouped together based on clinical similarities and uniform neuropathological features, including accumulation of lipofuscin in lysosomes and widespread gliosis. CLN7 disease is one of these NCLs that present in late infancy and is caused by mutations in the *CLN7/MFSD8* gene, which encodes a lysosomal membrane glycoprotein of unknown function, hence the biochemical processes affected by CLN7-loss of function are not understood. Here, we found in the *Cln7*$^{\Delta ex2}$ mouse model of CLN7 disease that failure in the autophagy-lysosomal pathway causes aberrant accumulation of reactive oxygen species (ROS)-producing brain mitochondria. Metabolic profile analysis of Cln7$^{\Delta ex2}$ neurons revealed a decrease in the basal oxygen consumption rate (OCR), ATP-linked and maximal OCR and proton leak, indicating bioenergetically impaired mitochondria. To assess the impact of ROS on CLN7 disease progression, Cln7$^{\Delta ex2}$ mice were crossed with mice expressing a mitochondrial-tagged form of catalase (mCAT) governed by a neuron-specific promoter (Cln7$^{\Delta ex2}$-CAMKIIa^{Cre}-mCAT). The increased mROS observed in Cln7$^{\Delta ex2}$ neurons was abolished in Cln7$^{\Delta ex2}$-CAMKIIa^{Cre}-mCAT neurons, verifying the efficacy of this approach. The brain mitochondrial swelling and mitochondrial cristae profile widening observed in Cln7$^{\Delta ex2}$ mice were abolished in Cln7$^{\Delta ex2}$-CAMKIIa^{Cre}-mCAT mice. Notably, Cln7$^{\Delta ex2}$ brain accumulation of subunit C-ATPase and lysosomal lipofuscin, as well as gliosis, which are hallmarks of the disease, were ameliorated in Cln7$^{\Delta ex2}$-CAMKIIa^{Cre}-mCAT mice. Altogether, these findings indicate that the generation of ROS by bioenergetically-impaired mitochondria in Cln7$^{\Delta ex2}$ neurons contributes to the histopathological symptoms of CLN7 disease.

NC79
Oxidative stress-triggered nuclear WRAP53 translocation promotes neuronal survival and functional recovery after stroke

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Ischemic stroke is the leading cause of neurologic death and long-term adult disability worldwide. Ischemia-induced oxidative stress compromises genome integrity, resulting in DNA damage. Failure of neurons to efficiently repair DNA double-strands breaks (DSB) contributes to cerebral damage and impairs functional recovery after stroke. However, the molecular machinery that regulates DNA repair in this neurological disorder remains unknown. Here we describe that WRAP53 (WD40 encoding RNA Antisense to p53), a scaffold protein implicated in telomere elongation and DNA repair in tumor cells, plays an essential role in neuronal survival after ischemia. We found that experimental ischemia, performed by oxygen and glucose deprivation (OGD), promoted oxidative stress-induced DSB in neurons. These events spatiotemporally correlated with WRAP53 upregulation and nuclear translocation to activate DSBs repair response. Mechanistically, OGD triggered a burst in reactive oxygen species that induced both DSB and translocation of WRAP53 to the nucleus to promote DNA repair, a pathway that was confirmed in an in vivo mouse model of stroke. Noticeably, nuclear translocation of WRAP53 occurred faster in OGD neurons expressing the *Wrap53* human nonsynonymous single-nucleotide polymorphism (SNP) rs2287499 (c.202C>G). Patients carrying this SNP showed less infarct volume and better functional outcome after stroke. These results provide a new signaling role for reactive oxygen species in the mechanism of DSB repair in neurons and unravel the role of WRAP53 to maintaining genome integrity and survival after stroke.
This work was funded by the Instituto de Salud Carlos III (PI18/00265 and RD16/0019/0018), European Regional Development Fund (FEDER), Ministerio de Ciencia en Innovación (SAF2017-90794-REDT), European Union's Horizon 2020 Research and Innovation Programme (grant agreement 686009), Junta de Castilla y León (IES007P17 and Escalera de Excelencia CLU-2017-03 Cofinanciado por el P.O. FEDER de Castilla y León), and Fundación Ramón Areces.

NC80
Regulation of the extracellular matrix glycoprotein Reelin by the transcription factor NRF2: A protective mechanism for the brain under oxidative stress

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Dysregulation of oxidative metabolism is associated with structural and functional changes in the extracellular matrix (ECM) that have been associated with the pathogenesis and progression of several neuropsychiatric and neurodegenerative disorders. A crucial protein of the ECM is Reelin, a large glycoprotein that plays essential roles the developing and adult brain. Reelin is cleaved at two well-defined sites that generate N-terminal (N-R2), central, and C-terminal fragments. The selective cleave by well-established specific proteases provides several signalling mechanisms in the brain parenchyma that are disrupted by redox alterations.
Transcription factor NRF2, nowadays considered the master regulator of antioxidant response, activates a battery of antioxidant and cytoprotective genes, and, therefore, it should impact on the ECM. Here, we studied if NRF2 might modify the maturation of Reelin by targeting the expression of genes encoding ECM proteases. We found that in cultured neurons and astrocytes the NRF2 activator sulforaphane reduces the cleavage of Reelin that generates the N-R2 fragment. This reduction correlates with a decrease in the transcript and protein levels of several ECM proteases. These effects were further studied in cortex and hippocampus of adult *Nrf2*-knockout mice, where we found an increase in the levels of the N-R2 fragment and the metalloproteinase ADAMATS-5 that is not observed in wild type mice.
The control of Reelin cleavage by NRF2 provides a new and very relevant layer of regulation for brain homeostasis.

NC81
Hydrogen peroxide induces neurite degeneration and its phenomenon relates to neurodegenerative disorders

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Background: The number of neurodegenerative disorders patients such as Alzheimer's (AD) and Parkinson's is gradually increased all over the world. There are more than 6 million dementia in Japan, and the ratio of AD is about 60%. Tau

phosphorylation and amyloid plaques are well known as one reason of the onset and progression of AD. However, fundamental mechanism of AD has not yet elucidated. Several lines of evidence have been demonstrated that reactive oxygen species (ROS) are deeply correlated with AD. Accumulated with oxidative damage in the brain accelerates with cognitive dysfunction and increases the risk of AD onset. In this study, we tried to find an early sign prior to the cell death.
Methods: N1E-115 and Neuro2a neuroblastoma cells were used all experiments. Hydrogen peroxide were used as an oxidative stress and optimized for induction of neurite degeneration. Neurite degeneration-related proteins were determined by proteome analysis and same proteins expressions were also checked by AD-transgenic mice brains.
Results: Treatment with a low concentration of hydrogen peroxide induces neurite degeneration such as beading formations. We determined six neurite degeneration related proteins by LC-MS/MS in isolated neurite of hydrogen peroxide-treated neurons. Some proteins expressions were significantly increased in AD-transgenic mouse brains.
Conclusion: These abnormal morphologies are also found in the several neurodegenerative disorders. The mechanism of the induction of ROS-induced neurite degeneration is a key factor the elucidate of the reason of AD onset. We are focusing on these determined proteins functions for apply for therapeutic target of AD.

NC82
Increased cellular level of Coenzyme Q10 during the differentiation of neuronal cells

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Background: Reduced form of coenzyme Q (CoQ), ubiquinol, is an important lipid soluble antioxidant. Coenzyme Q (CoQ) is also an essential lipid in the mitochondrial respiratory chain. Unlike other antioxidants, such as vitamin E and vitamin C, CoQ is synthesized in vivo. In mammalian cells, the biosynthesis of CoQ involves two metabolic sequences. First one is a formation of quinone moiety and second one is a formation of polyprenyl side chain. Quinone moiety is made from tyrosine and the polyprenyl side chain is synthesized from acetyl-CoA. Levels of CoQ in neuronal cells are paid much attention. Several neuronal diseases are reported to be caused by the mutation of the enzymes to synthesis CoQ. Plasma and platelets levels of CoQ10, especially its reduced form, are reported to be decreased in Parkinson's diseases. In this study, we aimed to elucidate the relationship between neuronal differentiation and CoQ levels. We measured changes in CoQ levels during neuronal differentiation.
Methods: PC12 cells and N1E-115 cells were used as a neuronal model. PC12 cells were cultured DMEM/F-12 medium. N1E-115 cells were cultured DMEM medium. PC12 cells were differentiated by adding NGF and hormones to serum-free medium. N1E-115 cells differentiated by changing to serum-free medium. HPLC was used to measure CoQ levels. Real-time PCR was used to measure gene expression levels.
Results and Discussion: The amount of CoQ9 when PC12 cells and N1E-115 cells differentiated was analyzed and compared to the percentage of free cholesterol (FC) produced from acetyl-CoA, as is CoQ. As a result, in both cells, the amount of CoQ9/FC after differentiation increased compared to that before differentiation. These results imply that CoQ9 level is increased during the differentiation of neuronal cells. The mechanism(s) underlie this increase will be also discussed in the presentation.

NC83
Effects of mycotoxins and carotenoids on the blood brain barrier oxidative stress and mitochondrial gene expression *in vitro*

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The blood brain barrier is a highly selective semipermeable structure able to discriminate molecules and to keep brain's homeostasis. Beauvericin, enniatins, ochratoxin A and zeralonone are mycotoxins with a wide toxicity profile towards animals and humans, while carotenoids are natural antioxidants found in vegetables and fruits such as pumpkins. Both of them are able to penetrate the blood brain barrier and alter different pathways at low doses, specifically the electron transport chain. Therefore, ECV304 cells were used as a blood brain barrier model to analyze: a) oxidative stress by H2-dichlorofluorescein diacetate probe; b) expression profile by qPCR of 16 key mitochondrial related genes: MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, MT-CO1, MT-CO3, MT-ATP6, MT-ATP8, UCP2, MT-RNR2, MRPL12, SRXN1, TXNIP, OSGIN1. ECV304 cells were differentiated for 9 days and treated during 2 h, always at the same individual concentration of mycotoxin (100 nM) and pumpkin (500 nM), individually and combined. Oxidative stress experiments reported major differences at longer exposure times and higher levels of reactive oxygen species were generally found when mixing carotenoids and mycotoxins than for the individual treatments. Interestingly, the mixture

of beauvericin, enniatins and pumpkin extract revealed the same level of oxidative stress as the control suggesting an equilibrium between these molecules and normal reactive oxygen species levels. Gene expression profile showed significant alterations for most genes and conditions with MT-RNR2 as the most downregulated gene and TXNIP as the most upregulated. In conclusion, exposure to these molecules can significantly alter mitochondrial activity even at low doses which could modify brain homeostasis and trigger damaging processes.
Keywords: beauvericin, enniatins, zearalenone, ochratoxin A, pumpkin, qPCR
Acknowledgements: This work was supported by Spanish Ministry of Science and Innovation national project (PID2019-108070RB-I00-ALI) and PhD grant (BES-2017-081328).

NC84
Transcriptomic changes after exposure to pumpkin extract in human epithelial cells of a blood brain barrier model

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C. maxima (var. *Delica*) is a particular variety of pumpkin known for its beneficial effects and its high content in carotenoids, which are natural antioxidants from plants and bioavailable to animals through food consumption. Numerous biological effects have been attributed to carotenoids, such as: improved immune response, promote anti-inflammatory and anti-tumor properties, reduce the risk of cardiovascular and chronic degenerative diseases and mainly protect cells from oxidative stress. These compounds, through the blood stream, reach and accumulate in different tissues, including the brain, after crossing the blood-brain barrier. Since carotenoids have been reported to modify different mitochondrial processes, the aim of this study is to determine changes in respiratory chain and antioxidant gene expression on differentiated ECV304 cells after exposure to pumpkin extract (*C. maxima*). Cells were treated during 24 hours at 5 different extract concentrations measured by the relation in beta-carotene content: 1.72×10^{-4} - 1.72×10^{-3}- 1.72×10^{-2} - 0.172 - 1.72 nM in DMSO 0.5% and this solvent concentration as control. qPCR analysis was performed on 15 mitochondria related genes: MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-CO1, MT-CO3, MT-ATP6, MT-ATP8, MT-RNR2, MRPL12, OSGIN1, SRXN1, TXNIP, UCP2, and S18 as reference gene. Results demonstrate that dietary carotenoids act at transcriptomic level, reporting at low concentrations an overall protective pattern on electron transport chain complex I, while at highest concentrations on MT-CO1, MT-ATP8, MRPL12, OSGIN1, SRXN1, TXNIP, UCP2. Nevertheless, our findings show dietary complements should be carefully assessed because, even at low concentrations, electron transport chain gene expression is altered.
Keywords: Electron Transport Chain; Blood Brain Barrier; neurodegenerative diseases; ECV304; qPCR.
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NC85
Alteration of cellular defensive response and a possible involvement of ferroptosis cell death in Rett syndrome disorder

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Rett Syndrome (RTT) is a rare neurodevelopmental disorder caused in 90% of the cases by mutations in the X-linked gene encoding for MeCP2, an important epigenetic regulator. Since oxidative stress seems to play a key role in RTT physiopathology, we decided to evaluate the cellular defensive response controlled by NRF2 and the glutathione (GSH) biosynthetic route and cycle using fibroblasts obtained from RTT patients.
RTT cells showed significantly elevated baseline expression of GCLC and GCLM transcripts, the catalytic and modulatory subunits of y- glutamylcysteine synthetase, which catalyzes the first step of GSH biosynthesis. However, after treatment with 4-hydroxynonenal (4HNE; 1, 5, and 10 μM) for 2 and 24h, RTT cells responded to the oxidant insult with a significant and dose-dependent decrease in GCLC mRNA levels, while for GCLM we observed a significant downregulation only with 10 μM 4HNE.

Then, we investigated enzymatic activity and gene expression of GSH peroxidase (GPX) and GSH reductase (GR), involved in GSH use and recycling, respectively. In basal condition, both enzymes showed a significant decreased activity in RTT cells compared to control. After stimulation, GPX activity increased in RTT, but to a lesser extent than in control cells. GR activity was inhibited by 5 and 10 μM 4HNE at 24h. Conversely, a significant upregulated GPX and GR gene expression was observed under basal condition and after 4HNE stimulation in RTT cells compared to control. Additionally, Western blot analysis indicated an alteration in the protein expression of GPX4, a key regulator of ferroptosis that inhibits ferroptotic cell death by preventing iron-dependent accumulation of toxic lipid reactive oxygen species.
Taken together, these data show alterations in GSH-related biosynthesis and cycle pathways, suggesting a possible increased vulnerability of RTT cells to ferroptotic cell death, an aspect that deserves further investigation.

NC86
Aberrant NF-κB / Nrf2 cross-talk in Rett syndrome

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Rett syndrome (RTT) is a severe and progressive neurodevelopmental disorder primarily affecting girls. About 95% of classic RTT is caused by mutations at the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2). RTT patients display neurologic hallmarks (*i.e.*, stereotyped hand movements, seizures, and autistic-like behavior), as well as broad-spectrum co-morbidities (*i.e.*, sleep disturbances, breathing, gastrointestinal and cardiac problems). To date, despite the progress in understanding the pathogenesis of RTT, the molecular mechanisms linking MeCP2 deficiency to all the symptoms of this complex pathology remain not fully understood. In the last decade, a perturbed redox homeostasis together with a chronic subclinical inflammatory status (defined as 'OxInflammation') were reported to play a key role in RTT pathogenesis.
Nuclear factor-κB (NF-κB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2), two master regulators of cellular responses to inflammation and oxidative stress, can interact through a range of complex molecular pathways.
The present study aimed at investigating the cross-talk among the NF-κB and Nrf2 pathways in primary fibroblasts isolated from skin biopsies of RTT patients and healthy subjects (CTR), that were treated with lipopolysaccharides (LPS).
Our preliminary results show an increased nuclear translocation of NF-κB subunit p65 in RTT cells in basal condition, with a slightly augmented response to LPS challenge, moreover the NF-κB-dependent interleukin 6 (IL-6) gene expression was higher in LPS-stimulated CTR cells, as compared to RTT. On the other hand, basal RTT fibroblasts seem to exhibit a trend toward increased nuclear localization of Nrf2, while LPS stimuli did not induce significant alterations.
We hypothesize that the presence of Nrf2 and NF-κB p65 into the nuclear compartment could be due to a tentative response to the prolonged OxInflammatory condition, which occurs in RTT cells. However, further experiments are needed to better elucidate the possible interplay between these transcription factors.

NC87
An antioxidant-enriched diet improves the behavior, immunity and oxidative stress in a mouse model of Alzheimer's Disease

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The oxidative stress (OS) has a key role in the pathophysiology of the Alzheimer's Disease (AD). The increase of OS as well as immune function impairment appears before the onset of the disease in peritoneal leukocytes of female triple-transgenic mice for AD (3xTgAD). Furthermore, the supplementation with thiolic antioxidants, which are precursors of glutathione, has proven that can improve the immunity and oxidative stress alterations showed by prematurely aging mice. Therefore, the aim of the present work was to prove if an antioxidant-enriched diet could improve the behavior, immunity and OS in 3xTgAD. 3xTgAD male and female mice received a standard diet (ADC) or an enriched diet with 0,1% of thioproline and n-acetylcysteine (ADD), while another group of non-transgenic mice (NC) received a standard diet. The supplementation started at 2.5 months of age. At 2, 4, 6, 9, 12 and 15 months of age, the peritoneal leukocytes

were collected and the anti-tumoral Natural killer activity (NK) and OS (xanthine oxidase activity, glutathione and lipid peroxidation levels) were measured. Also, at 6 months, the episodic memory (recognition object test), the spontaneous horizontal exploration (T maze) and anxiety levels (elevated plus maze) were analyzed. Diet supplementation was maintained until the natural death of the animals to obtain their life span. The results showed that the impairment in behavior, NK and OS, particularly in adulthood, in ADC were ameliorate by the antioxidant supplementation. Furthermore, the longevity of ADD mice, especially in male, increased, showing values similar to those in NC mice.
Keywords: Alzheimer's Disease, antioxidants, behavior, glutathione, immunity, mice.

NC88
Pilot study: contributing to AD diagnosis with a novel set of non-invasive biomarkers

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Alzheimer's disease (AD) is the most common form of dementia and is estimated to affect more than 46 million people around the world. The most studied biomarkers for AD diagnosis are β-amyloid deposits determined by positron emission tomography (PET), glucose consumption in the brain by PET, structural magnetic resonance imaging (MRI), and cerebrospinal fluid biomarkers such as β-amyloid, tau, and phosphorylated tau. However, these biomarkers present severe problems due to their invasive nature and costs. Consequently, the study of biomarkers in peripheral blood is still of great interest. In previous studies, we proposed a novel set of biomarkers composed of 4 proteins that can be measured in serum samples: RCAN1, clusterin, RAGE, and PKR.
This work aimed to validate the relative contribution of these previous proteins to the AD diagnosis in a new cohort and to assess the relevance of the contribution of a new parameter: malondialdehyde (MDA) as a biomarker of oxidative stress. For that purpose, we measured RCAN1, clusterin, RAGE, and PKR levels in serum and MDA levels in plasma from healthy individuals, patients with mild cognitive impairment (MCI), dementia, and AD. Furthermore, we compared this set contribution to AD diagnosis with current clinical parameters such as ApoE genotype and mini-mental state examination (MMSE) punctuation.
Preliminary results show that MDA and RCAN1 explain an important variability of the sample (56 %) by themselves, compared with the clinical parameters (60 %) in terms of individuals with AD or without AD (healthy, MCI and dementia). Moreover, this model explains up to 70% of the sample variability when healthy individuals are excluded from the study. The more variability explained, the better the model. These results suggest that MDA and RCAN1 could be useful as biomarkers from AD.
Keywords: Alzheimer's disease, RCAN1 and MDA

NC89
Altered inflammation and redox networks in the blood of patients with mild dementia

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Background: Neurodegenerative diseases are featured by low-grade inflammation and deregulated redox balance, extensively evidenced in the brain. However, the impact on peripheral blood leukocytes is still poorly understood despite its relevance as a potential source of biomarkers.
Aim**:** to characterize the expression profile of inflammation and redox genes in the blood of patients with mild dementia (MCI), and the impact of disease-specific medication.
Method: Peripheral blood was collected in PAXgene tubes from 38 controls and 38 patients with Alzheimer's type MCI, assessed by TAU and Abeta levels in cerebrospinal fluid. Gene expression was assessed by qRT-PCR using targeted arrays with 168 inflammation and redox genes. Patients were also investigated after one year of treatment with choline esterase inhibitors (Rivastigmine or Donepezil). Gene expression was also correlated with the volumetric analysis by MRI of various hippocampal segments. The inflammation and redox genes that exhibited altered expression were further evaluated in the blood of mice expressing combined tauopathy and amyloidopathy.

Results: Using several bioinformatics tools, a panel of seven inflammation genes and eight redox genes were identified as promising blood biomarkers in MCI patients. Some of these genes were up-regulated also in the animal model. Interestingly, one year of treatment with Rivastigmine and Donepezil normalized the expression of both inflammation and redox genes.
Conclusion: Blood leukocytes carry a pathological transcriptional fingerprint of inter-connected inflammation and redox alterations that might represent biomarkers for monitoring disease in MCI patients as well as drug response.
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NC90
Disruption of neurovascular coupling in a rodent model of vascular dementia – can we rescue it by nitrate supplementation?

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The functional and structural integrity of the brain is critically dependent of well-regulated delivery of energetic substrates matching the neuronal activity increase - neurovascular coupling (NVC). The NVC's dysfunction is an early event leading to neurodegeneration in several pathologies, including vascular dementia (VD). In view of the central role of nitric oxide (•NO) in NVC, it is hypothesized that altered •NO signaling and ensued impairment of cerebral perfusion have a prominent role in the dysfunctional cascade leading to neurodegeneration. We further hypothesize that nitrate, acting as a metabolic precursor of •NO, might improve NVC thus mitigating age-related cognitive impairment. Attempting to establish a proof of concept for this hypothesis and investigate the underlying mechanisms, in this work tested whether nitrate supplementation is able to circumventing altered •NO bioactivity, thereby rescuing the NVC functionality and cognitive function in a rodent model of VD.
The rodent model of VD consists in rats submitted to a permanent occlusion of common carotid arteries (2VO). The nitrate supplementation was provided in water for 8 weeks. After that period the different group of animals were tested in terms of memory performance (Barnes maze), functionally of the neurovascular coupling (laser Doppler flowmetry), nitrite/nitrate load (gas-phase chemiluminescence), vascular remodeling (MRA/IHC), inflammation and brain metabolism (MRS/IHC).
The 2VO rats display a significant decline of spatial memory along with an impaired NVC (both in hippocampus and somatosensory cortex) and significant alterations in cerebral vascular architecture and neuroinflammation. Remarkably, nitrate supplementation was able to mitigate the memory impairment promoted by chronic hypoperfusion, to modulate the hemodynamic response to glutamatergic activation and to hamper some of the pathological features elicited by 2VO.
Conclusions: Data support that nitrate supplementation rescues cognitive function in a rodent model of VD, likely via improved hemodynamic responses.
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NC91
A blueberry anthocyanin extract still reduces microglial activation after simulated digestion

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Microglial activation and therefore neuroinflammation are processes that are at the basis of the development of several brain disorders, such as Alzheimer and Parkinson diseases. Considering that current pharmacological options for these disorders are not curative, often bring limited results and lead to serious long-term side effects, it is urgent to find alternative and better targeted strategies effective in limiting brain diseases progression. Dietary polyphenols, being multifaceted compounds, able to modulate several important inflammatory cell signaling pathways, are envisaged as promising alternative therapeutical agents in the context of many brain disorders. However, it is known that most polyphenols suffer chemical rearrangements during gastrointestinal passage, with potential loss or gain of bioactivity, and this issue needs to be further scrutinized.

In this study, an anthocyanin-rich extract obtained from Portuguese blueberries was subjected to a simulated digestion and after careful chemical characterization, the potential of both non-digested and digested extracts to combat neuroinflammation was evaluated, using a microglia N9 cell line. Although the extracts have markedly different chemical composition, both were efficient in reducing the production of key inflammatory markers and reactive oxygen species. This protection was shown to be related to the suppression of nuclear factor kappa B (NF-kB) activation. These results demonstrate that the anthocyanin extract, after simulated digestion, can efficiently reduce microglial activation and can, therefore, assume a potentially relevant role in prevention of neuroinflammation-related brain disorders.
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NC92
Mitochondrial dysfunction fuels inflammasome machinery in Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is a multifactorial condition caused by the interaction of a genetic predisposition with a variety of external perturbations in prenatal and perinatal periods. However, detailed pathogenic processes of ASD still remain to be fully elucidated. A vicious cycle between disturbed redox homeostasis and altered immune response, with mitochondrial dysfunction as the central hub between both, appears clearly involved in ASD pathophysiology.
In our recent report, ASD primary fibroblasts showed mitochondrial alterations in morphology, bioenergetics and dynamics associated with a more pronounced pro-oxidative state and defective antioxidant defenses. Based on the evidence of the pivotal role played by mitochondria and reactive oxygen species (ROS) in the activation of inflammasome, a multiprotein complex that mediates proinflammatory responses, in this study we investigated components and function of the inflammasome machinery in the attempt to unveil a new possible molecular mechanism underlying ASD immune abnormalities. In unstimulated ASD fibroblasts, we found an altered protein expression of different constituents of the inflammasome system including NLRC4, AIM2, caspase-1, caspase-4 and gasdermin D. Moreover, priming with lipopolysaccharide (LPS) followed by stimulation with the danger signal adenosine triphosphate (ATP) induced significantly higher levels of caspase-1 (i.e. both pro- and active forms) in ASD cells. Concomitantly, a dose-dependent increase in both cytotoxicity and ROS production was observed in ASD fibroblasts primed with increasing concentrations of LPS and treated with ATP. Our findings indicate a possible upregulated expression and function of inflammasome machinery in ASD cells and uncover a new potential dysfunctional interaction linking redox and immune responses in this complicated neurodevelopmental disorder.

NC93
The beneficial effect of an anthocyanin-rich extract from Portuguese blueberries on behavioral, molecular and cellular alterations in prenatal valproic acid-induced mice model of autism

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Anthocyanins are pigments essentially found in fruits and vegetables. The increasing *in vitro*, *in vivo* and epidemiological studies focused on the beneficial health effects of anthocyanins have encouraged the consumption of anthocyanin-rich foods around the world. Besides their well-recognized role as natural food colorants, several authors describe anthocyanins and their metabolites as neuroprotective agents, mainly due to their antioxidant and anti-inflammatory activities. Here, we aimed to study the potential therapeutic effects of an anthocyanin-rich extract (ARE) obtained from Portuguese blueberries in autistic-like deficits, as well as to dissect the hypothetical influence of gut microbiota on Autism spectrum

disorder (ASD) context. ASD is a group of neurodevelopmental disorders characterized by social impairments, stereotypical behaviour and intestinal disturbances. Apart from the strong genetic basis of ASD, environmental factors can have a great impact on the development of autistic features in humans. Valproic acid (VPA) is an anti-epileptic drug and a well-known environmental risk factor in the development of autism. In the present study, pregnant BALB/c females were treated subcutaneously with a single dose of VPA (500 mg/kg) or saline on gestational day 12.5. Male offspring were orally treated with the ARE (30 mg/kg/day) or vehicle for three weeks, being subjected to behavioral tests and to biochemical and histopathological evaluation. Our preliminary results suggest that this anthocyanin-rich extract is able to ameliorate the behavioral impairments in VPA in utero-exposed males. Additionally, VPA-mediated abnormalities in brain, intestine and gut microbiota are likely to be reduced by this ARE, mainly via antioxidant and anti-inflammatory mechanisms. Overall, our work suggests that anthocyanins extracted from Portuguese blueberries could be efficient agents for alleviating autistic impairments through the modulation of microbiota-gut-brain communication.
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NC94
Regulation of the Nrf2-Notch1 axis in neurons: a role in neurogenesis

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The known network of genes targeted by Nrf2 is rapidly expanding and with it, the discovery of novel roles for the redox sensitive transcription factor Nrf2. Of these, a critical contribution to stem cell maintenance and fate has been revealed through its direct upregulation of stemness transcription factor Notch1. Canonical Notch1 signalling relies on its proteolytic cleavage by γ-secretase to liberate the Notch1 intracellular signalling fragment and its transactivation at CBF-1 DNA motif sites. We here describe for the first-time evidence of the Nrf2-Notch1 axis in neural lineage using the human neural cell line SH-SY5Y capable of neurogenesis. In undifferentiated SH-SY5Y cells, overexpression of Nrf2 or its endogenous activation with diethylmaleate (DEM) dose-dependently drives Notch1 transactivation as measured using reporter gene assays. Moreover, Nrf2 enhances Notch1 signalling events at CBF-1 sites in a γ-secretase-dependent manner, presumably due to an increased abundance of Notch1 presented at the cell surface membrane. In accord with this, transactivation of key Notch1 targets Hes1 and Hey1 were also increased. Interestingly, upon neuronal differentiation with retinoic acid (RA), the Nrf2-Notch1 axis is supressed supporting its proposed role in maintenance of the undifferentiated neuronal state. Our findings demonstrate the existence of the Nrf2-Notch1 axis in the human neural lineage with possible functional consequences for neurogenesis. (Email: emily.boorman@kcl.ac.uk)
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NC95
Discovery of Redox-stress Signaling Threshold (RST) and enlarged RST is beneficial to delay aging in *C. elegans*

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It is known that ROS play a dual role since it can be either beneficial or harmful at different concentrations to living systems. There seems to be a concentration threshold, which determines the transition from their advantageous to detrimental effects. If we increase the threshold on purpose, that is, to enlarge the range of ROS to play an advantageous role, it should be beneficial for individuals. Based on the C. elegans model, paraquat (PQ) was used as a redox stress challenge and starvation was used as a method to modulate the threshold. Lifespan of nematode was used as an indicator to evaluate the different effects of redox stress induced by paraquat (PQ) at different concentrations. It is found that 0.1 mM PQ was the optimum concentration for prolonging lifespan, and redox stress stronger than 0.75 mM PQ challenge was harmful to nematode survival. Comparing the change trend of the maximal lifespan of nematodes in the control group and starvation group under different PQ concentrations, we discovered the threshold changed from 0.75 mM to about 2 mM. This means that starvation during development significantly expanded the concentration range of ROS that played a

beneficial role and increased the threshold. Furthermore, the *C. elegans* with enlarged threshold by starvation during development present improved Redox-stress Response Capacity (RRC) and healthspan. We discovered that there is a turning point at which advantage effect of redox stress switched to detrimental effect. We named it as "Redox-stress Signaling Threshold (RST)" defined as the maximum level bearing redox stress as signaling benefit. Furthermore, we found that starvation during development could enlarge RST, indicating that this value is not fixed and can be enlarged by the adaptive response induced by early stress. And starvation during development to enlarge the threshold could improve RRC and healthspan. Enlarging the threshold value through early stimulation will be an effective strategy to delay aging.

NC96

The association of the glutathione redox state with colorectal cancer and its effectiveness as a tumor marker

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The role of oxidative stress in cancer is a matter of great interest due to the implication of reactive oxygen species (ROS) and their oxidation products in initiation of tumorogenesis, its progression and metastatic dissemination. Although a great effort has been made in order to identify the mechanisms of ROS-induced malignant cell transformation, the validation of oxidative stress byproducts as potential tumor markers remains to be established. By measuring the glutathione redox state in terms of oxidized (GSSG) to reduced (GSH) (GSSG/GSH x100) in the serum of colorectal cancer patients, we were able to identify significant changes compared with healthy subjects which are compatible with its effectiveness as a tumor marker. The thiol redox state showed a significant increased towards oxidation in the patient group and correlated significantly with both the tumor state and with their clinical evolution. The diagnostic tests (sensitivity, specificity, PPV, NPV and accuracy) of the glutathione levels and the classical tumor markers (TM) was studied, and their respective ROC curves were obtained. Statistical analysis shows a sensitivity and specificity of serum glutathione far above TM CEA and CA19.9 suggesting their usefulness for the diagnostic and monitoring of affected patients.
We concluded that the use of the GSSG/GSH ratio is an easy assay which can be validated as a novel clinical tumor marker for the diagnosis and monitoring of colorectal cancer.
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Reference: Delia Acevedo León, PhD Thesis 2019 University of Valencia.

NC97

Androgen-dependent prostate cancer cells reprogram their metabolic signature upon Glut-1 upregulation by Manganese Superoxide Dismutase (Mnsod/SOD2)

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Prostate cancer is the first cause of cancer in men in Europe. The prostate gland accounts for some unique metabolic characteristics, this causes that the metabolic features of prostate tumor are also unclear, and the knowledge gather in other tumour types is not applicable. The mitochondrial superoxide dismutase (Mnsod/SOD2) is the major redox regulator in the mitochondria and recently it has been proposed as a metabolic regulator.

Our work uses stable overexpressing MnSOD cell (on an androgen-dependent human prostate cancer cell line LNCaP), to study metabolic alterations upon MnSOD regulation. We also studied Balb/c transgenic mice overexpressing MnSOD systemically (n_{wt}=5, n_{SOD}=5) and a small subset of samples of redical prostatectomy from prostate cancer samples (n=5). Mnsod overexpression increases glucose transporter Glut1 and glucose uptake. This is not an insulin-mediated effect and seems to be sex-dependent, being present in male mice only. This event concurs with a series of substantialmetabolic rearrangements. A concomitant decrease in glycolytic and pentose phosphate activity and an increase in electron transfer in the mitochondrial electronic chain was observed. Metabolomic analysis showed an alteration of the Krebs Cycle in order to produce amino-acid intermediates by decreasing succinatedehydrogenase. This in turn generates a 13 time-increase in the oncometabolitesuccinate and seems to indicate a shortcut in the cycle. The energy sensor AMPK is decreased at basal protein levels and when its is phosphorylated levels in response to glucose deprivation, survival to glucose deprivation is improve in Mnsod-cells. Finally, results in prostate cancer patients indicate that glandular areas presenting high levels of Mnsod display high levels of Glut1 protein levels (R2=0.287 p-value<0.0001), indicating an analogous event in patients to those observed in cell culture and mice. Our works points out Mnsod as one major regulators for both redox and glycolytic metabolism in prostate cancer.

NC98
Sensitization of Melanoma Cells to Nitrosourea Treatment by Orchestrating Oxidative Stress and IKKβ Inhibition

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Nitrosourea represents one of the most active classes of chemotherapeutic alkylating agents for metastatic melanoma. Treatment with nitrosoureas, including fotemustine, caused severe systemic side effects which hamper its clinical use. IKKβ has been shown to be a target in enhancing the cytotoxic effects of anti-cancer drugs. Previous study showed that tumor cell death induced by nitrosourea can be increased by the upregulation of ROS production. Here, we investigate the potential therapeutic strategy for sensitizing the anti-tumor effects of nitrosourea alkylating agents using a ROS-inducing IKKβ inhibitor.
In melanoma cell lines, we found that SC-514, a ROS-inducing IKKβ inhibitor, enhanced cytotoxic effects of nitrosoureas. In A375 and G361 melanoma cells, SC-514/fotemustine combination induced concomitant DNA damage, cell cycle arrest and apoptosis. In A375 and G361 xenografts, SC-514 cooperated with nitrosourea to reduce tumor size and malignancy. Mechanism study revealed that elevating ROS level resulted in increased DNA crosslink efficiency triggered by nitrosoureas; anti-oxidant NAC significantly blocked the synergistic cell death action induced by SC-514 and fotemustine; and IKKβ inhibition enhances DNA damage signals and sensitizes fotemustine-induced cell death. To determine whether ROS would alter DNA crosslink *in vitro*, nitrosoureas-induced calf thymus DNA crosslink formation was analyzed and modified alkaline comet assay was used. We found that H_2O_2 increased the DNA-crosslinked fraction when the calf thymus DNA was exposed to nitrosoureas; and that SC-514 significantly enhanced DNA crosslink levels induced by fotemustine and treatment with NAC significantly rescued the SC-514/fotemustine-induced DNA crosslink. We further found that overexpression of constitutively active IKKβ rescued SC-514/fotemustine-mediated DNA damage and cell death. *In vivo*, SC-514 also increased fotemustine-induced DNA damage signaling. Taken together, our results illustrate a new direction in nitrosourea treatment, and reveal that the combination of ROS-inducing IKKβ inhibitors with nitrosoureas can be potentially exploited for melanoma therapy.
Keywords: Melanoma; Reactive oxygen species; IKKβ; Nitrosourea

NC99
δ-tocotrienol triggers ROS/MAPK axis-mediated apoptosis in ovarian cancer cells

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Ovarian cancer is one of the major causes of cancer-related death among women worldwide. Currently, its treatment is based on cytoreductive surgery followed by chemotherapy, which is unfortunately accompanied by severe toxicity and drug resistance development. Thereby, more effective and better-tolerated therapeutic approaches are urgently needed.
Tocotrienols (TTs) have recently shown great potential in ovarian cancer management. However, the molecular mechanisms underlying this potent antitumor activity are not clear. Here, we investigated the anticancer effects of δ-TT on IGROV-1 and SKOV-3 cells. We demonstrated that it could trigger cell cycle block at G1-S phase and mitochondrial apoptosis. In particular, we observed that the proapoptotic activity of δ-TT was associated with mitochondrial ROS generation and subsequent JNK and p38 phosphorylation. In conclusion, we found that δ-TT induces G1 phase cell cycle

arrest and ROS/MAPK-related apoptosis in ovarian cancer cells, thus representing a promising option for new chemopreventive/therapeutic strategies for ovarian cancer.

NC100

Relevance of mitochondrial dysfunction during Sorafenib-induced cell death in liver cancer cells. Role of oxidative and nitrogen reactive species

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Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the fourth most frequent cause of cancer-related death worldwide. Sorafenib, as tyrosine kinase inhibitor, is the still the first option for the treatment of patients at advanced stage of HCC. The low response rate is attributed to intrinsic resistance of HCC cells to Sorafenib. The present study showed that the sub-therapeutic dose of Sorafenib (10 nM) was associated with activation of caspase-9, AMP-activated protein kinase (AMPK), sustained autophagy, peroxisome proliferator-activated receptor-coactivator 1α (PGC-1α) and mitochondrial function in HepG2 cells. The increased mitochondrial respiration by Sorafenib (10 nM) was also observed in permeabilized HepG2 cells, but not in isolated rat mitochondria, which suggests the involvement of an upstream component in this regulatory mechanism. The basal glycolysis was dose dependently increased at early time point studied (6 hours). Differently, the administration of therapeutic dose of Sorafenib (10 μM, 24 hours) induced mitochondrial dysfunction, dropped basal glycolysis and promoted cell death. Although AMPK was upregulated, mitochondrial dysfunction by Sorafenib (10 μM) was associated with PGC-1α inhibition in HepG2 cells. The pattern of Sorafenib cytotoxicity was tightly related to increased superoxide anion generation and mitochondrial hyperpolarization of HepG2 cells. In this setting, the inhibition of nitric oxide generation by L-NAME increased superoxide anion production and further reduced mitochondrial respiration in HepG2. In conclusion, the accurate control of the administered dose of Sorafenib is relevant for the potential prosurvival or proapoptotic properties induced by the drug in liver cancer cells. Nitric oxide exerted a relevant role in preventing mitochondrial superoxide anion uncoupling and respiration in Sorafenib-treated liver cancer cells.

NC101

WIP induces oxidant tolerance in glioblastoma cells through NRF2/KEAP1 axis regulation

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Tumor cells exhibit both high proliferation and metabolic rates, which eventually produce exacerbated levels of reactive oxygen species (ROS) that must be controlled. Wiskott–Aldrich syndrome protein (WASP)-interacting protein (WIP) is a scaffold multifunctional protein essentially related to Actin polymerization regulation, involved in the formation of podosomes and invadopodia. These functions support the pro-tumoral role of WIP, endowing cancer cells with anchorage-independent growth and higher motility. Besides, WIP promotes cell survival and proliferation through poorly understood mechanisms. In this study, we have focused on a possible relation between WIP and redox homeostasis in glioblastomas. We show that the absence of WIP induced an increase of ROS levels, which correlated with a reduction of the levels of NRF2 (Nuclear factor (erythroid-derived 2)-like 2), master regulator of redox homeostasis. We demonstrate that WIP stabilizes NRF2 through the inhibition of E3 ligase adapter KEAP1, main NRF2 postranslational repressor, thus helping to maintain redox homeostasis. What is more, the overexpression of NRF2ΔETGE mutant, resistant to KEAP1 targeted degradation, in WIP depleted cells, restored normal ROS levels. Finally, we show that the mechanism underlying high KEAP1 (Kelch-like ECH-associated protein 1) activity in WIP-depleted cells consists in a WIP-dependent Actin cytoskeleton reorganization, which probably modifies the binding between KEAP1 and F-Actin.

Together, our results show a novel role of WIP in cancer development, through NRF2 activity regulation and the maintenance of oxidant tolerance in cancer cells, which could be addressed as novel target for the development of antitumoral therapies.

NC102

Generation of hydrogen peroxide in water irradiated by carbon-ion beam. Effects of dissolved oxygen and LET.

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The oxygen independent formation of hydrogen peroxide (H2O2) in an aqueous solution during carbon-ion beam irradiation was demonstrated. The radiation-induced hydroxyl radical (•OH) generation in an aqueous solution was reported to occur in two different localization densities, which were at the milli-molar (relatively sparse) and/or molar (super-dense) levels. In the milli-molar-level •OH generation atmosphere, •OH generated at a molecular distance of 4.3–6.6 nm are likely unable to interact each other. However, in the molar-level •OH generation atmosphere, several •OH were generated with a molecular distance of 1 nm or less, and two •OH can react to directly make H2O2.

An aliquot of ultra-pure water was irradiated by 290 MeV/nucleon carbon-ion beam at the Heavy-Ion Medical Accelerator in Chiba (HIMAC, NIRS/QST, Chiba, Japan). Irradiation experiments were performed under air or hypoxic (<0.5% oxygen) conditions, and several linear energy transfer (LET) conditions (20, 40, 60, 80, or >100 keV/μm). H2O2 generations in irradiated samples were estimated by three methods below. 1) A red quinoid dye (absorbance at 505 nm) formed by a reaction of 4-aminoantipyrine and phenol and H2O2 under coexisting peroxidase were measured using the spectrophotometer. 2) The •OH synthesized from H2O2 under the presence of Fe2+ was spin-trapped with DMPO, and the •OH adduct of DMPO (DMPO-OH) was then measured as an index of H2O2 using an X-band EPR. 3) The •OH synthesized from H2O2 under the UVB irradiation was spin-trapped with DMPO, and the DMPO-OH was then measured by an X-band EPR. Amounts of H2O2 generation per dose was estimated.

H2O2 generation under air condition, i.e. total H2O2 generation, decreased with LET increasing. H2O2 generation under hypoxic condition, i.e. oxygen independent H2O2 generation, increased with LET increasing. The oxygen dependent H2O2 generation, i.e. subtraction of H2O2 generation under hypoxic condition from H2O2 generation under air condition, decreased with LET increasing. Those results suggest that the super-dense •OH generation was increased with LET increasing. High LET beam could make H2O2 oxygen independently.

NC104

Effect of reaction media on the scavenging reaction of water-solubilized 2,2-diphenyl-1-picrylhydrazy radical by ascorbic acid

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Background: The radical-scavenging reaction of antioxidants is known to be significantly affected by pH, solvents, and metal ions. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical has been used to evaluate the radical-scavenging activity of antioxidants as a reactivity model of reactive oxygen species. However, the insolubility of the DPPH radical in water has precluded its use in aqueous media. Recently, we have succeeded in solubilizing the DPPH radical in water using β-cyclodextrin (β-CD). This enables us to evaluate the scavenging activity of the DPPH radical by antioxidants in aqueous media. We report herein the effect of reaction media on the scavenging reaction of the DPPH radical by ascorbic acid, a representative water-soluble antioxidant.
Methods: 25 mL of boiling water (Milli-Q) was added to the mixture containing the DPPH radical (0.15 mmol) and β-CD (0.70 mmol), and the suspension was cooled to room temperature. The filtration of the suspension by a membrane filter (pore size: 0.22 μm) gave a deep violet solution of the DPPH radical. The rates of the scavenging reaction of the DPPH radical were determined by monitoring the absorbance change due to the DPPH radical using a stopped-flow technique on a UNISOKU RSP-1000-02NM spectrophotometer.

Results: The second-order rate constant (k) of the reaction between ascorbic acid and the β-CD-solubilized DPPH radical was determined to be 5.4×10^3 M^{-1} s^{-1} in phosphate buffer (0.05 M, pH 7.0) at 25 °C. The k value increased with increasing the pH value. Furthermore, magnesium perchlorate significantly accelerated this reaction. On the other hand, when water was replaced by deuterium oxide to prepare the phosphate buffer (0.05 M, pD 7.0), the k value significantly decreased to be 1.5×10^3 M^{-1} s^{-1}.
Conclusion: The scavenging reaction of the DPPH radical by ascorbic acid is significantly affected by reaction media.
Keywords: Antioxidant, ascorbic acid, radical, kinetics, stopped-flow

NC105
Therapeutic potential of nitric oxide released by a robust NO carrier based on a porous Ti-MOF

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Background: Delivery vehicles capable to store, transport and release nitric oxide (NO) have been exploited for several biomedical applications. However, due to NO short biological lifetime, instability and potential toxicity is challenging address these limitations and guarantee the target delivery of therapeutic NO amounts. Herein, a titanium-based metal-organic framework (MOF) is proposed as a new NO carrier, designed with a specific porous framework that allow NO to be stored by adsorption [1].
Methods: Ti-MOF was prepared according to the reported procedure[2]. The gravimetric method was used to record the NO kinetic adsorption profiles and NO release profiles were evaluated under biological conditions using NO electrochemical sensor and Griess assay. The potential therapeutic application was demonstrated through the control of mitochondrial respiration in HeLa cells using an oxygen electrode and through the stimulation of cell migration using the Oris™ Cell Migration Assay.
Results: This material has demonstrated its superiority by featuring a set of key properties required for such application never achieved by other porous materials, namely: (1) high NO storage capacity (3 μmol mg^{-1}_{solid}), (2) excellent biocompatibility over several cell lines, (3) high stability under biological conditions (< 9% degradation in 72 hours) and (4) slow release of pure NO in biological medium (2 hours for 90% release). Moreover, the NO adsorption/release mechanism is new for this type of materials and is based on the formation of complexed nitrites on the inorganic building unit of the Ti-MOF, conferring its slow release mechanism.NO-released by this new donor actively regulate cells O_2 consumption in a dose-dependent manner and promote the cell migration, achieving an improvement in wound closure of 8.4±1.4% after 24 hours.
Conclusion: These results provide a step forward in the development of advanced nanoporous materials for NO delivery for use in wound healing therapy.
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NC106
Alteration of redox state following radon inhalation depends on the antioxidant capacity of organs

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Radiation induces the production of reactive oxygen species (ROS). Previously, we reported that low-dose X-irradiation or radon inhalation (α-ray emitter) activates antioxidative functions in mouse organs due to the production of small amounts of ROS. Since antioxidant capacity in organs varies, a moderate radiation dose for the activation of antioxidative functions may depend on organs. Therefore, in this study, we evaluated the relationship among superoxide dismutase (SOD), catalase (CAT), total glutathione (GSH), hydrogen peroxide (H_2O_2), and lipid peroxide (LPO) in the brain, lung, heart, liver, stomach, pancreas, kidney, small intestine, and large intestine in mice following radon inhalation. Mice inhaled radon at concentrations of 2 or 20 kBq/m^3 for 1, 3, or 10 d. Scatter plots were used to evaluate the redox state by conducting principal component analysis (PCA) of the data obtained from control mice subjected to sham inhalation. Furthermore, we calculated correlation coefficients in each cluster obtained following radon inhalation. The results showed that the liver and kidney had high antioxidant capacity. The brain, pancreas, and stomach had low antioxidant capacities and lipid peroxide (LPO) content; however, the lungs, heart, small intestine, and large intestine had high LPO content but low antioxidant capacities. The correlation coefficients associated with GSH, H_2O_2, and LPO in most organ groups were changed following radon inhalation. Although correlation coefficients related to SOD in organs with a low antioxidant capacity were also changed, no changes were observed in the correlation coefficients in organs with high antioxidant capacity. These findings suggested that radon inhalation could alter the redox state in organs; however, its characteristics were dependent on the total antioxidant capacity of the organs.

NC107
Low-dose irradiation reduces forced swim test-induced immobility and oxidative stress in mice

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The forced swim test (FST), a screening test that evaluates anti-depressant activity, causes immobility and induces oxidative stress. In our previous study, inhalation of radon, an α-ray emitter, prevented and alleviated FST-induced depression-like symptoms in mice. After mice were irradiated at 0.1 or 0.5 Gy of X-ray (dose rate: 1.2 Gy/m) or 0.1 or 0.5 Gy of γ-ray (dose rate: 0.6 or 3.0 Gy/h, 7 days), they were forced to swim in a columned container with a diameter of 10 cm and a height of 25 cm for 10 minutes on 5 consecutive days. The water temperature was maintained at 25°C ± 1°C. The immobility time was measured by three observers who were blinded to the intervention process. The results showed that 0.1 Gy (X-ray; high-dose rate) irradiation inhibited the immobility whereas 0.5 Gy (γ-ray; low-dose rate) inhibited the immobility. The FST decreased catalase activity in the brain, suggesting FST-induced oxidative stress. However, the catalase activity in the brains of mice that received γ-ray irradiation increased in a dose-dependent manner, whereas lipid peroxide levels in the brains of mice that received X-ray irradiation decreased dose-dependent matter. In addition, there were no significant differences in superoxide dismutase, catalase, and total glutathione levels between the sham and irradiation groups. These findings indicate that irradiation at a high-dose rate is effective in inhibiting FST-induced immobility and oxidative stress in mice.

NC108
Redox regulation of autophagy by thioredoxin *o*1 and its involvement in tobacco BY-2 cell viability under oxidative conditions

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Autophagy is a process by which damaged or non-useful components are degraded in a lytic cell compartment for recycling or elimination. Autophagy is essential for physiological function and stress defense of plant cells although the mechanisms involved in its regulation are less known than in animal systems. Redox regulation of autophagy components is emerging as a key mechanism and thioredoxins (TRXs) have been proposed as regulators. In a previous work, we described that mitochondrial/nuclear PsTRX*o*1 had a protective role increasing cell viability and delaying cell death after treating over-expressing PsTRX*o*1 tobacco BY-2 cells with H_2O_2 (Ortiz-Espín et al., 2015, Ann. Bot. 116:571-), however the link of TRX with the autophagy process was not studied. In this work, the *in vitro* interaction of autophagy related protein ATG4 and TRX*o*1 is shown by dot-blot trap analysis and the redox regulation of its activity by the thioredoxin system (NADPH/thioredoxin reductase/thioredoxin *o*1) is demonstrated. Moreover, taking into account all these results, we hypothesize an additional functional role for TRX*o*1 and autophagy in the oxidative stress response of the over-expressing TBY-2 cells, collaborating to increase cell viability. For that, we analyze ATG4 and ATG8 expression by

qPCR, western blot as well as ATG4 activity in parallel to cell viability, autolysosomes visualization and immunolocalization of ATG8 by fluorescence microscopy using known autophagy inhibitors. The results indicate that overexpression of PsTRX*o*1 could be influencing the establishment of an autophagic process in the response of TBY-2 cells to H_2O_2, collaborating in the observed increased cell survival. The TRX*o*1 role could be through the regulation of key target proteins as ATG4. [Supported by Seneca Foundation (Excellence 19876/GERM/15) and MEC-FEDER, Spain (BFU2017-86585-P). SB-V, OL-V and MCM were supported by MEC-FPI, AECID (México) and MICINN-Ramón y Cajal, respectively. Authors acknowledge the technical support of Sandra Correa in microscopy analysis.

NC109
In vitro cytoprotective effect and antioxidant capacity of salmon, mackerel and herring hydrolysates in Caco-2 clone (TC7) cells

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The fish frame contains remarkable amounts of muscle proteins. To date, the use of fractionation of heads, backbones or other fish organs in the production of protein supplements there is not, or only very limited. Thus, protein hydrolysates are a good candidate as food ingredients for nutritional supplement. In this study, the *in vitro* cytotoxicity of salmon, mackerel and herring hydrolysates was evaluated by MTT essay in the concentration range from 1 to 1:32 dilution in Human colon adenocarcinoma cells (Caco-2/TC7). The induction of oxidative stress, as a possible mechanism of toxicity, was determined by lipid peroxidation (LPO) and reactive oxygen species (ROS) generation. The protective effect of fish hydrolysates against oxidative stress was evaluated using H_2O_2-stressed human intestinal differentiated Caco-2/TC7 cells. All hydrolysates showed a hormetic effect when these cells were exposed to 1:16 dilution, preventing a decrease in cell viability. Pure hydrolysates decreased the LPO production in these cells. The highest cytoprotective effect was obtained with HSV hydrolysate with 2.5-fold. The intracellular reactive oxygen species (ROS) accumulation induced by H_2O_2 was suppressed by all pure hydrolysates. Due to the importance of the bioavailability of hydrolysates, their *in vitro* gastrointestinal digestion in Caco-2/TC7 cell were carried out. The viability of bioavailable fraction was compared with pure hydrolysates. The results suggest that HMH, HSV, HSB, HMB, HSH and Collagen have an adequate bioavailability due to the bioavailable fraction of each hydrolysate shows the same viability as pure hydrolysate. So, all hydrolysates were non-cytotoxic and prevented the propagation of oxidative stress by LPO and ROS generation in Caco-2/TC7 cell. Thus, they can be beneficial ingredients with antioxidant properties and can have protective effects against ROS mediated intestinal injuries.
Keywords: fish hydrolysates, cytotoxicity, oxidative stress, cytoprotective effect, bioavailability

NC110
Detection of active cell death markers in rehydrated lichen thalli and the involvement of nitrogen monoxide (NO)

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Lichen desiccation/rehydration cycles lead to an increased oxidative stress modulated by the multifaceted mediator nitrogen monoxide (NO). Active cell death, frequently triggered by oxidative damage with NO participation, has been confirmed even in unicellular organisms. This adaptive mechanism has not been studied in lichens and no specific experimental protocols exist. Hoechst 33342 enters viable cells and DNA binding increases its fluorescence, particularly intense in condensed apoptotic chromatin. YO-PRO-1 can only permeate the altered membrane of apoptotic P2X7-positive cells. Proteolytic caspases are activated upon different types of active cell death. Our objectives are to determine if these markers indicate active cell death in *Ramalina farinacea* after desiccation/rehydration and to study the effect of NO scavenging. YO-PRO-1, Hoechst 33342 and Caspase 3/7 Green DNA binding were assessed in thalli rehydrated with deionized water and with a cocktail of apoptosis inducers. A 24 h kinetics and a microscopical analysis were performed. YO-PRO-1 fluorescence was not detected, Hoechst 33342 staining abruptly decreases during the first hours, while caspase-like activity associated to phycobionts steadily increases. Whereas the apoptosis inducers cocktail 1x significantly increased caspase-like activity affecting both symbionts, Hoechst staining was only affected at 10x. NO scavenging diminishes caspase-like activation and seems to accelerate Hoechst abrupt decrease during thallus rehydration. In conclusion, the demonstration of caspase-like activity in *R. farinacea* and its Trebouxia phycobionts point to the presence of active cell death but other methods assessing cell effective death or DNA irreversible fragmentation (i.e. TUNEL assay) are necessary to confirm this feature.

Keywords: Apoptosis, Caspases, Hoechst 33342, Oxidative stress, Programmed cell death and YO-PRO-1.

NC111
Immunomodulation of melanoma in vitro and *in vivo* using reactive oxygen species

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Background: Immunogenic cancer cell death (ICD) delivers inflammatory stimuli to elicit anti-tumor immunity. Release of find-me (HMGB1, ATP) and eat-me (calreticulin; CRT) signals enables dendritic cell maturation and presentation of tumor antigen to T-cells. Effective ICD-inducing therapies are anthracyclines, ionizing irradiation, and photodynamic therapies. Intriguingly, these antitumor strategies show concomitant generation of reactive oxygen species (ROS) that can drive cell death and immunogenic signaling responses. In cancer cells, the importance of ROS is underappreciated in the onset of ICD.
Methods: We employed a novel antitumor modality capable of releasing a several types of tumor-toxic ROS simultaneously.
Results: *In vitro*, these ROS were able to eradicate melanoma cells in an immunogenic fashion. This was shown by ATP release, CRT upregulation, and MHC class I upregulation. In a syngeneic mouse melanoma model, we observed a decrease of melanoma growth with multi-ROS therapy. Intriguingly, the immunogenicity of this therapy was shown by vaccinating mice with ROS-killed tumor cells. Upon later re-challenge with viable melanoma cells, only a fraction of the tumor had grown, underlining the immunogenicity of ROS-induced cell death. By contrast, injection with mitomycin C (MMC) killed melanoma cells did not protect mice from subsequent injection with viable cells. As expected, injection with the highly immunogenic drug mitoxantrone (MTX) conferred superior protection in the vaccination experiment. Activation of lymph node-derived T-cells was best in MTX mice, followed by the cells from the plasma group, and was lowest in cells from the MMC group.
Conclusion: Not only the event of cell death but also its auxiliary signals are important in the formation of anti-tumor immune responses. ROS play a significant role in this process and may add to our understanding of the importance of reactive oxygen species in oncology.
Keywords: Immunogenic cancer cell death; medical gas plasma technology

NC112
Inhibiting Bach1 enhanced the activation of Nrf2 signaling and the degradation of HNE in response to oxidative stress

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Exposure to air pollution nanoparticles (APN) increases neurodegeneration and accelerates cognitive decline in normal aging or Alzheimer's disease (AD). One putative mechanism is that the older or AD patients are more susceptible to APN neurotoxicity is due to accelerated accumulation of 4-hydroxynonenal (HNE). Upon HNE exposure, cells usually can upregulate its detoxification through the activation of Nrf2 signaling. With aging however, the activation of Nrf2 signaling in response to oxidative stress declines, this would sensitize older individuals to oxidative damage. Bach1 is a suppressor of Nrf2 signaling and was involved in aging-related loss of Nrf2 signaling. In current study we further examined the effect of inhibiting Bach1 on Nrf2 signaling and detoxification of HNE. HNE (HNE-protein conjugates) was increased with aging in various tissues of mice including the brain. Upon exposure to APN, HNE was increased in the lungs of the old (21-month) but not young adult (6-month) mice. In addition, APN increased HNE in the cortex of young AD transgenic but not wild type mice. These data suggest that young adults have an efficient capacity to eliminate HNE but it declines with aging or in AD. Knockout (by siRNA) or inhibition (by heme or ZnPP) of Bach1 increased the basal expression of Nrf2-target antioxidant genes (GCLC, GCLM, NQO1, HO-1 and GSTA4-4) and enhanced the induction of these genes by APN or sulforaphane in human bronchial epithelia cells and mouse microglia BV2 cells. Meanwhile, Bach1 knockout/inhibition increased the degradation of HNE and significantly reduced HNE-protein conjugates caused by APN in microglia. In summary, HNE accumulation upon APN exposure was exacerbated in the older adults, and inhibiting bach1 enhanced Nrf2 activation and HNE detoxification upon oxidative stress, suggesting that Bach1 is a potential target to reverse age-related decline in Nrf2 activation and induction of HNE detoxification in response to oxidative stress. This project was supported by NIH grants R01-ES023864, R01-AG051521, and P01-AG055367.

NC113
Apolipoprotein E4 and oxidative stress: A prospective study

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Introduction: Apolipoprotein E4 (APOE4) is a major genetic risk factor for the development of Alzheimer's disease (AD). Our previous study found that young healthy APOE4 carriers present a reductive stress, with lower oxidized products. However, as oxidative damage is involved in AD pathophysiology, this reductive stress may not be sustained over time. Therefore, we aimed to analyze the oxidative status of these healthy APOE4 carriers after 10 years.
Methods: Blood samples were collected from 39 healthy adults aged 35-64 that participated in the previous study 10 years ago. Subjects were grouped by genotype: 3/3 (15), 3/4 (14) and 4/4 (10). Whole blood levels of oxidized (GSSG) and reduced (GSH) glutathione were measured by spectrophotometry and GSSG/GSH ratio was calculated. Lipid peroxidation, determined as malondialdehyde (MDA) formation from peroxides were measured by high performance liquid chromatography.
Results: Current measures show no significant difference in glutathione levels or ratio between the three groups. APOE4 carriers (3/4 and 4/4) have significantly higher levels of MDA when compared to non-carriers (3/3).
Conclusions: The reductive stress seen in younger APOE4 was carriers reverted with time. Furthermore, they now present oxidative damage.

NC114
The SGAs Olanzapine and Aripiprazole inhibit mitochondrial respiration and induce oxidative stress

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The chronic intake the antipsychotic (SGAs) Olanzapine (Ola) is related with obesity and increased incidence of cardiovascular diseases (CVD). In contrast, the more recently developed antipsychotics like Aripiprazole (Ari) do not induce weight gain and are expected to have a safer CV profile. We aimed to analyze the impact of by Ola and Ari on mitochondrial activity and its potential link to their metabolic and CV secondary effects. First, we isolated liver mitochondria from C57BL/6 mice ip injected with Ari or Ola at 5mg/kg for 2 weeks and found that both Ari and Ola accumulated in the mitochondrial membranes, with Ola showing a trend for higher levels but also faster turnover. We next tested the effect of 3 μM Ari or Ola on mitochondrial respiration *in vitro* in bovine aortic endothelial cells (BAEC), and we found a significant inhibition of both basal and maximal respiration following 3 h of treatment with both Ari and Ola. However, at 24 h of treatment respiration levels had recovered in Ola but not in Ari treated cells. To confirm the relevance of these results, we analyzed mitochondrial respiration in PBMC isolated from blood samples of healthy volunteers 5 h after the administration of Ari or Ola. We observed that while Ola main effect was the increase in reserve capacity, Ari reduced ATP-linked respiration, increased proton-leak and reduced coupling efficiency, suggesting a more toxic effect of Ari also in humans. We next evaluated the impact on mitochondrial superoxide production by MitoSOX staining of BAEC cells, we found that after 3 h of treatment, 0.3 μM Ola, and 3 μM Ari, increased MitoSOX staining. The toxicity of this effect was evaluated by western blot analysis of 4-HNE modified proteins. The results showed that after 6 h of treatment with 3 μM of both Ari and Ola increased the levels of 4-HNE modified proteins. Finally, we tested the effect of long-term treatment on respiration treating mice with Ari and Ola (5 mg/kg) for 6 months and analyzing oxygen consumption rates using metabolic chambers. We found that, both Ari and Ola treatment decreased respiration rates but the effect was detected earlier in Ari treated mice, being significant after 1 month of treatment, a change not associated with any significant reduction in activity nor food intake. These results suggest that, both Ola and Ari (0.3-3 μM) can interfere with mitochondrial respiration by accumulating in mitochondrial membranes, and increase mitochondrial superoxide production and cellular oxidative stress. But, long term treatment data both *in vivo* and *in vitro* suggest that Ari has even more prevailing toxic effects on mitochondrial respiration. Since mitochondrial dysfunction plays a key role in CVD these results could challenge the current view that Ari may be more safe from a CV point of view because it does not induce significant weight gain nor has the sedative effects that Ola has.

NC115

Antagonistic regulation of apoptosis by polysulfides and thioredoxin via persulfidation of caspases

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Hydrogen sulfide is involved in a large number of physiological processes including cell survival and death, prompting investigation into its mechanisms of action and therapeutic potential. Recent research suggests that the cellular effects of hydrogen sulfide are mediated in part by sulfane sulfur species including persulfides and polysulfides. We investigated the apoptosis-modulating effects of polysulfides, in particular, in relation to the caspase cascade that mediates the intrinsic apoptotic pathway. Our analyses revealed that polysulfides strongly and rapidly inhibited the enzymatic activity of purified caspase-3, which was due to persulfidation of its catalytic cysteine. Moreover, polysulfides triggered the persulfidation and deactivation of cleaved caspae-3 under apoptotic conditions in HeLa cells. The results also suggested a role for the thioredoxin/thioredoxin reductase system (Trx/TrxR) in caspase depersulfidation. Trx/TrxR restored the activity of polysulfide-inactivated caspase-3 *in vitro*, and inhibition of TrxR potentiated polysulfide-mediated suppression of caspase-3 activity *in situ*. Furthermore, under conditions of low TrxR activity, early cell exposure to polysulfides lead to enhanced persulfidation of initiator caspase-9, resulting in decreased apoptosis. Our investigation also revealed that the proenzymes, procaspase-3/-9, are basally persulfidated in unstimulated cells and become depersulfidated during their processing and activation. Inhibition of TrxR attenuated the depersulfidation and activation of caspase-9. Taken together, these findings suggest that reversible persulfidation of caspase-3/-9 participates in apoptosis regulation.